(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 April 2002 (11.04.2002)

PCT

(10) International Publication Number
WO 02/29106 A2

(51) International Patent Classification[7]: C12Q 1/68

(21) International Application Number: PCT/US01/30933

(22) International Filing Date: 2 October 2001 (02.10.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/237,938 3 October 2000 (03.10.2000) US
60/237,937 3 October 2000 (03.10.2000) US

(71) Applicant: **CALIFORNIA INSTITUTE OF TECHNOLOGY** [US/US]; 1200 East California Boulevard, Pasadena, CA 91125 (US).

(72) Inventors: **CHOU, Hou-Pu**; 231 Acalanes Drive, #2, Sunnyvale, CA 94086 (US). **EYAL, Shulamit**; 229 South Wilson Avenue, #4, Pasadena, CA 91106 (US). **FU, Anne, Y.**; 9 Calle Cameron, San Clemente, CA 92673 (US). **QUAKE, Stephen, R.**; 744 Plymouth Road, San Marino, CA 91108 (US).

(74) Agents: **TOBIN, Kent, J.** et al.; Townsend and Townsend and Crew, LLP, Two Embarcadero Center, 8th Floor, San Francisco, CA 94111-3834 (US).

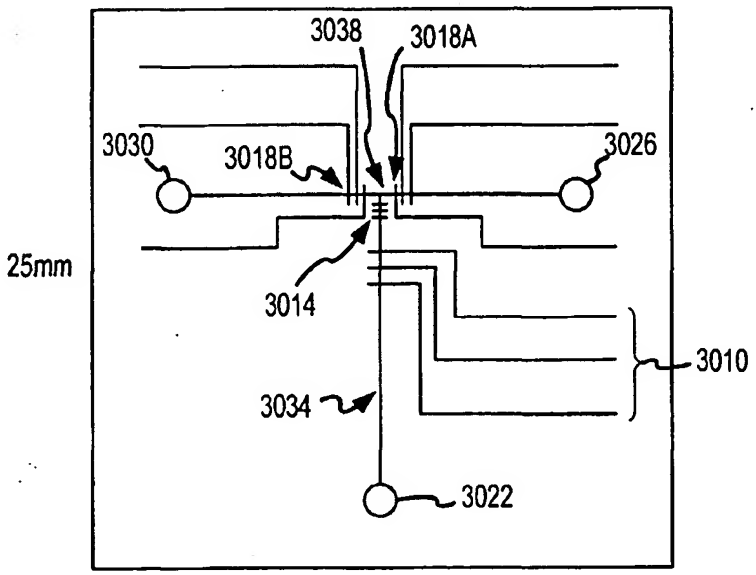
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

[Continued on next page]

(54) Title: MICROFLUIDIC DEVICES AND METHODS OF USE



(57) Abstract: A microfluidic device comprises pumps, valves, and fluid oscillation dampers. In a device employed for sorting, an entity is flowed by the pump along a flow channel through a detection region to a junction. Based upon an identity of the entity determined in the detection region, a waste or collection valve located on opposite branches of the flow channel at the junction are actuated, thereby routing the entity to either a waste pool or a collection pool. A damper structure may be located between the pump and the junction. The damper reduces the amplitude of oscillation pressure in the flow channel due to operation of the pump, thereby lessening oscillation in velocity of the entity during sorting process. The microfluidic device may be formed in a block of elastomer material, with thin membranes of the elastomer material deflectable into the flow channel to provide pump or valve functionality. Velocity independent cytometry methods and apparatuses are also described.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Microfluidic Devices and Methods of Use

CROSS-REFERENCE TO RELATED APPLICATION(S)

The instant nonprovisional patent application claims priority from United States Provisional Patent Application No. 60/237,938, entitled MICROFLUIDIC DEVICES AND METHODS OF USE, filed October 3, 2000. The instant nonprovisional patent application also claims priority from United States Provisional Patent Application No. 60/237,937, entitled VELOCITY INDEPENDENT MICROFLUIDIC FLOW CYTOMETRY, filed October 3, 2000. These provisional patent applications are incorporated by reference herein for all purposes.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

Work described herein has been supported, in part, by National Institute of Health grant HG-01642-02. The United States Government may therefore have certain rights in the invention.

BACKGROUND OF THE INVENTION

Sorting of objects based upon size is extremely useful in many technical fields. For example, many assays in biology require determination of the size of molecular-sized entities. Of particular importance is the measurement of length distribution of DNA molecules in a heterogeneous solution. This is commonly done using gel electrophoresis, in which the molecules are separated by their differing mobility in a gel matrix in an applied electric field, and their positions detected by absorption or emission of radiation. The lengths of the DNA molecules are then inferred from their mobility.

While powerful, electrophoretic methods pose disadvantages. For medium to large DNA molecules, resolution, i.e. the minimum length difference at which different molecular lengths may be distinguished, is limited to approximately 10% of the total length. For extremely large DNA molecules, the conventional sorting procedure is not workable. Moreover, gel electrophoresis is a relatively lengthy procedure, and may require on the order of hours or days to perform.

The sorting of cellular-sized entities is also an important task. Conventional flow cell sorters are designed to have a flow chamber with a nozzle and are

based on the principle of hydrodynamic focusing with sheath flow. Most conventional cell sorters combine the technology of piezo-electric drop generation and electrostatic deflection to achieve droplet generation and high sorting rates. However, this approach offers some important disadvantages. One disadvantage is that the complexity, size, and expense of the sorting device requires that it be reusable in order to be cost-effective. Reuse can in turn lead to problems with residual materials causing contamination of samples and turbulent fluid flow.

Another disadvantage of conventional sorting technologies is an inability to readily integrate sorting with other activities. For example, due to the mechanical complexity of conventional sorting apparatuses, pre-sorting activities such as labeling and post-sorting activities such as crystallization are typically performed on different devices, requiring physical transfer of the pre- and post-sorted sample to the sampling apparatus.

This transfer requires precise and careful handling in order to prevent any loss of the frequently small volumes of sample involved. Moreover, sample handling for conventional sorter structures is time-consuming. The resulting delay may hinder analysis of materials having limited lifetimes, or prevent sorting that is based upon time-dependent criteria.

Therefore, there is a need in the art for a simple, inexpensive, and easily fabricated integratable sorting device which relies upon the mechanical control of fluid flow rather than upon electrical interaction between the particle and the solute.

SUMMARY OF THE INVENTION

An embodiment of a microfluidic device in accordance with the present invention comprises pumps, valves, and fluid oscillation dampers. In a device employed for sorting, an entity is flowed by the pump along a flow channel through a detection region to a junction. Based upon an identity of the entity determined in the detection region, a waste or collection valve located on opposite branches of the flow channel is actuated, thereby routing the entity to either a waste pool or a collection pool. A damper structure may be located between the pump and the junction. The damper reduces the amplitude of oscillation pressure in the flow channel due to operation of the pump, thereby lessening oscillation in velocity of the entity during sorting process. The microfluidic device may be formed in a block of elastomer material, with thin membranes of the elastomer material deflectable into the flow channel to provide pump or valve functionality.

An embodiment of a microfluidic device in accordance with the present invention comprises a flow channel, a pump operatively interconnected to said flow channel for moving a fluid in said flow channel, and a damper operatively interconnected to said flow channel for reducing the fluid oscillation in said flow channel.

An embodiment of a microfluidic sorting device comprises a first flow channel formed in a first layer of elastomer material, a first end of the first flow channel in fluid communication with a collection pool and a second end of the first flow channel in fluid communication with a waste pool. A second flow channel is formed in the first elastomer layer, a first end of the second flow channel in fluid communication with an injection pool and a second end of the second flow channel in fluid communication with the first flow channel at a junction. A collection valve is adjacent to a first side of the junction proximate to the collection pool, the collection valve comprising a first recess formed in a second elastomer layer overlying the first elastomer layer, the first recess separated from the first flow channel by a first membrane portion of the second elastomer layer deflectable into the first flow channel. A waste valve is adjacent to a second side of the junction proximate to the waste pool, the waste valve comprising a second recess formed in the second elastomer layer separated from the second flow channel by a second membrane portion of the second elastomer layer deflectable into the first flow channel. A pump adjacent to a third side of the junction proximate to the injection pool, the pump comprising at least three pressure channels formed in the second elastomer layer and separated from second flow channel by third membrane portions of the second elastomer layer deflectable into the second flow channel. A detection region is positioned between the injection pool and the junction, one of an open and closed state of the collection valve and the waste valve determined by an identity of a sortable entity detected in the detection region.

An embodiment of a damper in accordance with the present invention for a microfluidic device comprises a flow channel formed in an elastomer material, and an energy absorber adjacent to the flow channel and configured to absorb an energy of oscillation of a fluid positioned within the flow channel.

An embodiment of a sorting method in accordance with the present invention comprises deflecting a first elastomer membrane of an elastomer block into a flow channel to cause a sortable entity to flow into a detection region positioned upstream of a junction in the flow channel. The detection region is interrogated to identify the sortable entity within the detection region. Based upon an identity of the sortable entity,

one of a second membrane and a third membrane of the elastomer block are deflected into one of a first branch flow channel portion and a second branch flow channel portion respectively, located downstream of the junction. This causes the sortable entity to flow to one of a collection pool or a waste pool.

An embodiment of a method for dampening pressure oscillations in a flow channel comprises providing an energy absorber adjacent to the flow channel, such that the energy absorber experiences a change in response the pressure oscillations.

These and other embodiments of the present invention, as well as its advantages and features, are described in more detail in conjunction with the text below and attached figures.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is an illustration of a first elastomeric layer formed on top of a micromachined mold.

Fig. 2 is an illustration of a second elastomeric layer formed on top of a micromachined mold.

Fig. 3 is an illustration of the elastomeric layer of Fig. 2 removed from the micromachined mold and positioned over the top of the elastomeric layer of Fig. 1

Fig. 4 is an illustration corresponding to Fig. 3, but showing the second elastomeric layer positioned on top of the first elastomeric layer.

Fig. 5 is an illustration corresponding to Fig. 4, but showing the first and second elastomeric layers bonded together.

Fig. 6 is an illustration corresponding to Fig. 5, but showing the first micromachined mold removed and a planar substrate positioned in its place.

Fig. 7A is an illustration corresponding to Fig. 6, but showing the elastomeric structure sealed onto the planar substrate.

Figs. 7B is a front sectional view corresponding to Fig. 7A, showing an open flow channel.

Figs. 7C-7G are illustrations showing steps of a method for forming an elastomeric structure having a membrane formed from a separate elastomeric layer.

Fig. 8A and 8B illustrates valve opening vs. applied pressure for various flow channels.

Fig. 9 illustrates time response of a 100μm×100μm×10μm RTV microvalve.

Fig. 12A is a top schematic view of an on/off valve.

Fig. 12B is a sectional elevation view along line 23B-23B in Fig. 12A

Fig. 13A is a top schematic view of a peristaltic pumping system.

Fig. 13B is a sectional elevation view along line 24B-24B in Fig. 13A

Fig. 14 is a graph showing experimentally achieved pumping rates vs. frequency for an embodiment of the peristaltic pumping system of Fig. 13.

Fig. 15A is a top schematic view of one control line actuating multiple flow lines simultaneously.

Fig. 15B is a sectional elevation view along line 26B-26B in Fig. 15A

Fig. 16 is a schematic illustration of a multiplexed system adapted to permit flow through various channels.

Fig. 17A is a plan view of a flow layer of an addressable reaction chamber structure.

Fig. 17B is a bottom plan view of a control channel layer of an addressable reaction chamber structure.

Fig. 17C is an exploded perspective view of the addressable reaction chamber structure formed by bonding the control channel layer of Fig 17B to the top of the flow layer of Fig 17A.

Fig. 17D is a sectional elevation view corresponding to Fig. 17C, taken along line 28D-28D in Fig. 17C.

Fig. 18 is a schematic of a system adapted to selectively direct fluid flow into any of an array of reaction wells.

Fig. 19 is a schematic of a system adapted for selectable lateral flow between parallel flow channels.

Fig. 20A is a bottom plan view of first layer (i.e.: the flow channel layer) of elastomer of a switchable flow array.

Fig. 20B is a bottom plan view of a control channel layer of a switchable flow array.

Fig. 20C shows the alignment of the first layer of elastomer of Fig. 20A with one set of control channels in the second layer of elastomer of Fig. 20B.

Fig. 20D also shows the alignment of the first layer of elastomer of Fig. 20A with the other set of control channels in the second layer of elastomer of Fig. 20B.

Figs. 21A-21J show views of one embodiment of a normally-closed valve structure in accordance with the present invention.

Figs. 22A and 22B show plan views illustrating operation of one embodiment of a side-actuated valve structure in accordance with the present invention.

Figure 23 shows a cross-sectional view of one embodiment of a composite structure in accordance with the present invention.

Figure 24 shows a cross-sectional view of another embodiment of a composite structure in accordance with the present invention.

Figure 25 shows a cross-sectional view of another embodiment of a composite structure in accordance with the present invention.

Fig. 26 is a cross-sectional view of one embodiment of a damper structure in accordance with the present invention.

Fig. 27 is a cross-sectional view of an alternative embodiment of a damper structure in accordance with the present invention.

Fig. 28 is a plan view of another alternative embodiment of a damper structure in accordance with the present invention.

Figs. 29A-B are cross-sectional views of operation of one embodiment of a circulation apparatus including a damper structure in accordance with the present invention.

Fig. 30A is a schematic plan view of a cell sorter structure in accordance with one embodiment of the present invention.

Fig. 30B is a photograph of a plan view of the cell sorter shown in Fig. 30A.

Figs. 31A and 31B are schematic views of a T-junction of a sorter structure in accordance with an embodiment of the present invention engaged in reversible sorting.

Fig. 32A plots cell velocity versus pump frequency for one embodiment of a cell sorter in accordance with the present invention.

Fig. 32B plots mean reversible time versus pump frequency for the embodiment of a cell sorter of Fig. 32A.

Fig. 33A plots optical intensity over time for the cell sorter structure of Fig. 33C operated at first frequency.

Fig. 33B plots optical intensity over time for the cell sorter structure of Fig. 33C operated at first frequency.

Fig. 33C is a schematic view of a cell sorter structure in accordance with an alternative embodiment in accordance with the present invention.

Fig. 34 plots flow velocity versus pump frequency for cell sorters fabricated from different elastomeric materials.

Fig. 35A is a fluorescence signal illustration showing that at a similar flow velocity the peak intensity and the peak area is proportional to the length of the DNA being detected by flow cytometry.

Fig. 35B is a fluorescence signal illustration showing peak areas of two similar length DNAs with different flow velocity.

Fig. 36A shows a schematic illustration of an apparatus of the present invention.

Fig. 36B shows CCD camera image of fluorescein solution flowing through a T-channel of a microfluidic device illuminated by the scanning beam and two-dimensional laser beam scanning by acousto-optic modulator and the corresponding square wave (top, i.e., Ch1) and fluorescence data (bottom, i.e., Ch2).

Fig. 36C illustrates a laser beam location and the order after passing through two orthogonally located acousto-optic modulators.

Fig. 37 is an overlay graph of fluorescence peaks from two detection zones and normalized fluorescence peaks.

Fig. 38 shows the velocity as a function of time of an experiment with two kinds of fluorescent beads.

Fig. 39 shows histogram of the velocities of 0.2 μm fluorescent beads that were in the system (solid line) and the corresponding theoretical one (dash line).

Fig. 40 shows histogram of λ DNA, where the center peak represents λ DNA (48 kbp) and the small peak on the right represents pairs of hybridized λ (96 kbp).

Fig. 41 shows a flow chart of a computer program for recording square waves and fluorescent peak data.

Fig. 42 shows a flow chart of a computer program for determining a velocity independent flow cytometry.

DESCRIPTION OF SPECIFIC EMBODIMENTS

I. Microfabrication Overview

The following discussion relates to formation of microfabricated fluidic devices utilizing elastomer materials, as described generally in U.S. patent applications 09/826,585 filed April 6, 2001, 09/724,784 filed November 28, 2000, and 09/605,520, filed June 27, 2000. These patent applications are hereby incorporated by reference.

1. Methods of Fabricating

Exemplary methods of fabricating the present invention are provided herein. It is to be understood that the present invention is not limited to fabrication by one or the other of these methods. Rather, other suitable methods of fabricating the present microstructures, including modifying the present methods, are also contemplated.

Figs. 1 to 7B illustrate sequential steps of a first preferred method of fabricating the present microstructure, (which may be used as a pump or valve). Figs. 8 to 18 illustrate sequential steps of a second preferred method of fabricating the present microstructure, (which also may be used as a pump or valve).

As will be explained, the preferred method of Figs. 1 to 7B involves using pre-cured elastomer layers which are assembled and bonded. In an alternative method, each layer of elastomer may be cured "in place". In the following description "channel" refers to a recess in the elastomeric structure which can contain a flow of fluid or gas.

Referring to Fig. 1, a first micro-machined mold 10 is provided. Micro-machined mold 10 may be fabricated by a number of conventional silicon processing methods, including but not limited to photolithography, ion-milling, and electron beam lithography.

As can be seen, micro-machined mold 10 has a raised line or protrusion 11 extending therealong. A first elastomeric layer 20 is cast on top of mold 10 such that a first recess 21 will be formed in the bottom surface of elastomeric layer 20, (recess 21 corresponding in dimension to protrusion 11), as shown.

As can be seen in Fig. 2, a second micro-machined mold 12 having a raised protrusion 13 extending therealong is also provided. A second elastomeric layer 22 is cast on top of mold 12, as shown, such that a recess 23 will be formed in its bottom surface corresponding to the dimensions of protrusion 13.

As can be seen in the sequential steps illustrated in Figs. 3 and 4, second elastomeric layer 22 is then removed from mold 12 and placed on top of first elastomeric layer 20. As can be seen, recess 23 extending along the bottom surface of second elastomeric layer 22 will form a flow channel 32.

Referring to Fig. 5, the separate first and second elastomeric layers 20 and 22 (Fig. 4) are then bonded together to form an integrated (i.e.: monolithic) elastomeric structure 24.

As can been seen in the sequential step of Figs. 6 and 7A, elastomeric structure 24 is then removed from mold 10 and positioned on top of a planar substrate 14. As can be seen in Fig. 7A and 7B, when elastomeric structure 24 has been sealed at its bottom surface to planar substrate 14, recess 21 will form a flow channel 30.

The present elastomeric structures form a reversible hermetic seal with nearly any smooth planar substrate. An advantage to forming a seal this way is that the elastomeric structures may be peeled up, washed, and re-used. In preferred aspects, planar substrate 14 is glass. A further advantage of using glass is that glass is transparent, allowing optical interrogation of elastomer channels and reservoirs. Alternatively, the elastomeric structure may be bonded onto a flat elastomer layer by the same method as described above, forming a permanent and high-strength bond. This may prove advantageous when higher back pressures are used.

As can be seen in Fig. 7A and 7B, flow channels 30 and 32 are preferably disposed at an angle to one another with a small membrane 25 of substrate 24 separating the top of flow channel 30 from the bottom of flow channel 32.

In preferred aspects, planar substrate 14 is glass. An advantage of using glass is that the present elastomeric structures may be peeled up, washed and reused. A further advantage of using glass is that optical sensing may be employed. Alternatively, planar substrate 14 may be an elastomer itself, which may prove advantageous when higher back pressures are used.

The method of fabrication just described may be varied to form a structure having a membrane composed of an elastomeric material different than that forming the walls of the channels of the device. This variant fabrication method is illustrated in Figs. 7C-7G.

Referring to Fig. 7C, a first micro-machined mold 10 is provided. Micro-machined mold 10 has a raised line or protrusion 11 extending therealong. In Fig. 7D, first elastomeric layer 20 is cast on top of first micro-machined mold 10 such that the top

of the first elastomeric layer 20 is flush with the top of raised line or protrusion 11. This may be accomplished by carefully controlling the volume of elastomeric material spun onto mold 10 relative to the known height of raised line 11. Alternatively, the desired shape could be formed by injection molding.

In Fig. 7E, second micro-machined mold 12 having a raised protrusion 13 extending therealong is also provided. Second elastomeric layer 22 is cast on top of second mold 12 as shown, such that recess 23 is formed in its bottom surface corresponding to the dimensions of protrusion 13.

In Fig. 7F, second elastomeric layer 22 is removed from mold 12 and placed on top of third elastomeric layer 222. Second elastomeric layer 22 is bonded to third elastomeric layer 20 to form integral elastomeric block 224 using techniques described in detail below. At this point in the process, recess 23 formerly occupied by raised line 13 will form flow channel 23.

In Fig. 7G, elastomeric block 224 is placed on top of first micro-machined mold 10 and first elastomeric layer 20. Elastomeric block and first elastomeric layer 20 are then bonded together to form an integrated (i.e.: monolithic) elastomeric structure 24 having a membrane composed of a separate elastomeric layer 222.

When elastomeric structure 24 has been sealed at its bottom surface to a planar substrate in the manner described above in connection with Fig. 7A, the recess formerly occupied by raised line 11 will form flow channel 30.

The variant fabrication method illustrated above in conjunction with FIGS. 7C-7G offers the advantage of permitting the membrane portion to be composed of a separate material than the elastomeric material of the remainder of the structure. This is important because the thickness and elastic properties of the membrane play a key role in operation of the device. Moreover, this method allows the separate elastomer layer to readily be subjected to conditioning prior to incorporation into the elastomer structure. As discussed in detail below, examples of potentially desirable condition include the introduction of magnetic or electrically conducting species to permit actuation of the membrane, and/or the introduction of dopant into the membrane in order to alter its elasticity.

While the above method is illustrated in connection with forming various shaped elastomeric layers formed by replication molding on top of a micromachined mold, the present invention is not limited to this technique. Other techniques could be employed to form the individual layers of shaped elastomeric material that are to be

bonded together. For example, a shaped layer of elastomeric material could be formed by laser cutting or injection molding, or by methods utilizing chemical etching and/or sacrificial materials as discussed below in conjunction with the second exemplary method.

An alternative method fabricates a patterned elastomer structure utilizing development of photoresist encapsulated within elastomer material. However, the methods in accordance with the present invention are not limited to utilizing photoresist. Other materials such as metals could also serve as sacrificial materials to be removed selective to the surrounding elastomer material, and the method would remain within the scope of the present invention. For example, gold metal may be etched selective to RTV 615 elastomer utilizing the appropriate chemical mixture.

2. Layer and Channel Dimensions

Microfabricated refers to the size of features of an elastomeric structure fabricated in accordance with an embodiment of the present invention. In general, variation in at least one dimension of microfabricated structures is controlled to the micron level, with at least one dimension being microscopic (i.e. below 1000 μm). Microfabrication typically involves semiconductor or MEMS fabrication techniques such as photolithography and spincoating that are designed for to produce feature dimensions on the microscopic level, with at least some of the dimension of the microfabricated structure requiring a microscope to reasonably resolve/image the structure.

In preferred aspects, flow channels 30, 32, 60 and 62 preferably have width-to-depth ratios of about 10:1. A non-exclusive list of other ranges of width-to-depth ratios in accordance with embodiments of the present invention is 0.1:1 to 100:1, more preferably 1:1 to 50:1, more preferably 2:1 to 20:1, and most preferably 3:1 to 15:1. In an exemplary aspect, flow channels 30, 32, 60 and 62 have widths of about 1 to 1000 microns. A non-exclusive list of other ranges of widths of flow channels in accordance with embodiments of the present invention is 0.01 to 1000 microns, more preferably 0.05 to 1000 microns, more preferably 0.2 to 500 microns, more preferably 1 to 250 microns, and most preferably 10 to 200 microns. Exemplary channel widths include 0.1 μm, 1 μm, 2 μm, 5 μm, 10 μm, 20 μm, 30 μm, 40 μm, 50 μm, 60 μm, 70 μm, 80 μm, 90 μm, 100 μm, 110 μm, 120 μm, 130 μm, 140 μm, 150 μm, 160 μm, 170 μm, 180 μm, 190 μm, 200 μm, 210 μm, 220 μm, 230 μm, 240 μm, and 250 μm.

Flow channels 30, 32, 60, and 62 have depths of about 1 to 100 microns. A non-exclusive list of other ranges of depths of flow channels in accordance with embodiments of the present invention is 0.01 to 1000 microns, more preferably 0.05 to 500 microns, more preferably 0.2 to 250 microns, and more preferably 1 to 100 microns, more preferably 2 to 20 microns, and most preferably 5 to 10 microns. Exemplary channel depths include including 0.01 μm, 0.02 μm, 0.05 μm, 0.1 μm, 0.2 μm, 0.5 μm, 1 μm, 2 μm, 3 μm, 4 μm, 5 μm, 7.5 μm, 10 μm, 12.5 μm, 15 μm, 17.5 μm, 20 μm, 22.5 μm, 25 μm, 30 μm, 40 μm, 50 μm, 75 μm, 100 μm, 150 μm, 200 μm, and 250 μm.

The flow channels are not limited to these specific dimension ranges and examples given above, and may vary in width in order to affect the magnitude of force required to deflect the membrane as discussed at length below in conjunction with Fig. 27. For example, extremely narrow flow channels having a width on the order of 0.01 μm may be useful in optical and other applications, as discussed in detail below. Elastomeric structures which include portions having channels of even greater width than described above are also contemplated by the present invention, and examples of applications of utilizing such wider flow channels include fluid reservoir and mixing channel structures.

The Elastomeric layers may be cast thick for mechanical stability. In an exemplary embodiment, elastomeric layer 22 of Fig. 1 is 50 microns to several centimeters thick, and more preferably approximately 4 mm thick. A non-exclusive list of ranges of thickness of the elastomer layer in accordance with other embodiments of the present invention is between about 0.1 micron to 10 cm, 1 micron to 5 cm, 10 microns to 2 cm, 100 microns to 10 mm.

Accordingly, membrane 25 of Fig. 7B separating flow channels 30 and 32 has a typical thickness of between about 0.01 and 1000 microns, more preferably 0.05 to 500 microns, more preferably 0.2 to 250, more preferably 1 to 100 microns, more preferably 2 to 50 microns, and most preferably 5 to 40 microns. As such, the thickness of elastomeric layer 22 is about 100 times the thickness of elastomeric layer 20. Exemplary membrane thicknesses include 0.01 μm, 0.02 μm, 0.03 μm, 0.05 μm, 0.1 μm, 0.2 μm, 0.3 μm, 0.5 μm, 1 μm, 2 μm, 3 μm, 5 μm, 7.5 μm, 10 μm, 12.5 μm, 15 μm, 17.5 μm, 20 μm, 22.5 μm, 25 μm, 30 μm, 40 μm, 50 μm, 75 μm, 100 μm, 150 μm, 200 μm, 250 μm, 300 μm, 400 μm, 500 μm, 750 μm, and 1000 μm.

3. Soft Lithographic Bonding

Preferably, elastomeric layers are bonded together chemically, using chemistry that is intrinsic to the polymers comprising the patterned elastomer layers. Most preferably, the bonding comprises two component "addition cure" bonding.

In a preferred aspect, the various layers of elastomer are bound together in a heterogenous bonding in which the layers have a different chemistry. Alternatively, a homogenous bonding may be used in which all layers would be of the same chemistry. Thirdly, the respective elastomer layers may optionally be glued together by an adhesive instead. In a fourth aspect, the elastomeric layers may be thermoset elastomers bonded together by heating.

In one aspect of homogeneous bonding, the elastomeric layers are composed of the same elastomer material, with the same chemical entity in one layer reacting with the same chemical entity in the other layer to bond the layers together. In one embodiment, bonding between polymer chains of like elastomer layers may result from activation of a crosslinking agent due to light, heat, or chemical reaction with a separate chemical species.

Alternatively in a heterogeneous aspect, the elastomeric layers are composed of different elastomeric materials, with a first chemical entity in one layer reacting with a second chemical entity in another layer. In one exemplary heterogenous aspect, the bonding process used to bind respective elastomeric layers together may comprise bonding together two layers of RTV 615 silicone. RTV 615 silicone is a two-part addition-cure silicone rubber. Part A contains vinyl groups and catalyst; part B contains silicon hydride (Si-H) groups. The conventional ratio for RTV 615 is 10A:1B. For bonding, one layer may be made with 30A:1B (i.e. excess vinyl groups) and the other with 3A:1B (i.e. excess Si-H groups). Each layer is cured separately. When the two layers are brought into contact and heated at elevated temperature, they bond irreversibly forming a monolithic elastomeric substrate.

In an exemplary aspect of the present invention, elastomeric structures are formed utilizing Dow Corning Sylgard 182, 184 or 186, or aliphatic urethane diacrylates such as (but not limited to) Ebecryl 270 or Irr 245 from UCB Chemical.

In one embodiment in accordance with the present invention, two-layer elastomeric structures were fabricated from Dow Corning Sylgard 184. The layer containing the flow channels had an A:B ratio of 20:1 was spin coated at 5000 rpm. The layer containing the control channels had an A:B ratio of 5:1

In another embodiment in accordance with the present invention, two-layer elastomeric structures were fabricated from pure acrylated Urethane Ebe 270. A thin bottom layer was spin coated at 8000 rpm for 15 seconds at 170°C. The top and bottom layers were initially cured under ultraviolet light for 10 minutes under nitrogen utilizing a Model ELC 500 device manufactured by Electrolite corporation. The assembled layers were then cured for an additional 30 minutes. Reaction was catalyzed by a 0.5% vol/vol mixture of Irgacure 500 manufactured by Ciba-Geigy Chemicals. The resulting elastomeric material exhibited moderate elasticity and adhesion to glass.

In another embodiment in accordance with the present invention, two-layer elastomeric structures were fabricated from a combination of 25% Ebe 270 / 50% Irr245 / 25% isopropyl alcohol for a thin bottom layer, and pure acrylated Urethane Ebe 270 as a top layer. The thin bottom layer was initially cured for 5 min, and the top layer initially cured for 10 minutes, under ultraviolet light under nitrogen utilizing a Model ELC 500 device manufactured by Electrolite corporation. The assembled layers were then cured for an additional 30 minutes. Reaction was catalyzed by a 0.5% vol/vol mixture of Irgacure 500 manufactured by Ciba-Geigy Chemicals. The resulting elastomeric material exhibited moderate elasticity and adhered to glass.

Alternatively, other bonding methods may be used, including activating the elastomer surface, for example by plasma exposure, so that the elastomer layers/substrate will bond when placed in contact. For example, one possible approach to bonding together elastomer layers composed of the same material is set forth by Duffy et al, "Rapid Prototyping of Microfluidic Systems in Poly (dimethylsiloxane)", Analytical Chemistry (1998), 70, 4974-4984, incorporated herein by reference. This paper discusses that exposing polydimethylsiloxane (PDMS) layers to oxygen plasma causes oxidation of the surface, with irreversible bonding occurring when the two oxidized layers are placed into contact.

Yet another approach to bonding together successive layers of elastomer is to utilize the adhesive properties of uncured elastomer. Specifically, a thin layer of uncured elastomer such as RTV 615 is applied on top of a first cured elastomeric layer. Next, a second cured elastomeric layer is placed on top of the uncured elastomeric layer. The thin middle layer of uncured elastomer is then cured to produce a monolithic elastomeric structure. Alternatively, uncured elastomer can be applied to the bottom of a first cured elastomer layer, with the first cured elastomer layer placed on top of a second

cured elastomer layer. Curing the middle thin elastomer layer again results in formation of a monolithic elastomeric structure.

Bonding together of successive elastomer layers in accordance with embodiments of the present invention need not result in a monolithic elastomeric structure. Successive layers of different elastomer materials can be bonded together to form an embodiment of a structure in accordance with the present invention. For example General Electric RTV and Dow Corning Sylgard 184 can be bonded together to form a multilayer structure.

Moreover, bonding together of successive elastomer layers in accordance with the present invention need not be permanent. In certain embodiments, the strength of the bond between elastomer layers need only maintain contact and resist separation under the forces encountered during membrane actuation. Application of greater force will cause the elastomer layers to separate, for example allowing flushing and reuse of flow or control channels present in the separated layers.

Where encapsulation of sacrificial layers is employed to fabricate the elastomer structure, bonding of successive elastomeric layers may be accomplished by pouring uncured elastomer over a previously cured elastomeric layer and any sacrificial material patterned thereupon. Bonding between elastomer layers occurs due to interpenetration and reaction of the polymer chains of an uncured elastomer layer with the polymer chains of a cured elastomer layer. Subsequent curing of the elastomeric layer will create a bond between the elastomeric layers and create a monolithic elastomeric structure.

Referring to the first method of Figs. 1 to 7B, first elastomeric layer 20 may be created by spin-coating an RTV mixture on microfabricated mold 12 at 2000 rpm's for 30 seconds yielding a thickness of approximately 40 microns. Second elastomeric layer 22 may be created by spin-coating an RTV mixture on microfabricated mold 11. Both layers 20 and 22 may be separately baked or cured at about 80°C for 1.5 hours. The second elastomeric layer 22 may be bonded onto first elastomeric layer 20 at about 80°C for about 1.5 hours.

Micromachined molds 10 and 12 may be patterned photoresist on silicon wafers. In an exemplary aspect, a Shipley SJR 5740 photoresist was spun at 2000 rpm patterned with a high resolution transparency film as a mask and then developed yielding an inverse channel of approximately 10 microns in height. When baked at approximately

200°C for about 30 minutes, the photoresist reflows and the inverse channels become rounded. In preferred aspects, the molds may be treated with trimethylchlorosilane (TMCS) vapor for about a minute before each use in order to prevent adhesion of silicone rubber.

4. Suitable Elastomeric Materials

Allcock et al, Contemporary Polymer Chemistry, 2nd Ed. describes elastomers in general as polymers existing at a temperature between their glass transition temperature and liquefaction temperature. Elastomeric materials exhibit elastic properties because the polymer chains readily undergo torsional motion to permit uncoiling of the backbone chains in response to a force, with the backbone chains recoiling to assume the prior shape in the absence of the force. In general, elastomers deform when force is applied, but then return to their original shape when the force is removed. The elasticity exhibited by elastomeric materials may be characterized by a Young's modulus. Elastomeric materials having a Young's modulus of between about 1 Pa – 1 TPa, more preferably between about 10 Pa – 100 GPa, more preferably between about 20 Pa – 1 GPa, more preferably between about 50 Pa – 10 MPa, and more preferably between about 100 Pa – 1 MPa are useful in accordance with the present invention, although elastomeric materials having a Young's modulus outside of these ranges could also be utilized depending upon the needs of a particular application.

The systems of the present invention may be fabricated from a wide variety of elastomers. In an exemplary aspect, the elastomeric layers may preferably be fabricated from silicone rubber. However, other suitable elastomers may also be used.

In an exemplary aspect of the present invention, the present systems are fabricated from an elastomeric polymer such as GE RTV 615 (formulation), a vinyl-silane crosslinked (type) silicone elastomer (family). However, the present systems are not limited to this one formulation, type or even this family of polymer; rather, nearly any elastomeric polymer is suitable. An important requirement for the preferred method of fabrication of the present microvalves is the ability to bond multiple layers of elastomers together. In the case of multilayer soft lithography, layers of elastomer are cured separately and then bonded together. This scheme requires that cured layers possess sufficient reactivity to bond together. Either the layers may be of the same type, and are capable of bonding to themselves, or they may be of two different types, and are capable

of bonding to each other. Other possibilities include the use an adhesive between layers and the use of thermoset elastomers.

Given the tremendous diversity of polymer chemistries, precursors, synthetic methods, reaction conditions, and potential additives, there are a huge number of possible elastomer systems that could be used to make monolithic elastomeric microvalves and pumps. Variations in the materials used will most likely be driven by the need for particular material properties, i.e. solvent resistance, stiffness, gas permeability, or temperature stability.

For example, a diluent may be included during formation of the elastomer material to alter its properties. In one embodiment, a diluent is added to the elastomer comprising the membrane layer to lessen the stiffness of the membrane and thereby reduce the actuation force required. Two examples of diluent for elastomer materials are General Electric SF96, and DMV-V21 manufactured by Gelest, Inc. of Tullytown, Pennsylvania. In general, the diluent is mixed with the elastomer at a ratio of between about 15% and 30%.

There are many, many types of elastomeric polymers. A brief description of the most common classes of elastomers is presented here, with the intent of showing that even with relatively "standard" polymers, many possibilities for bonding exist. Common elastomeric polymers include polyisoprene, polybutadiene, polychloroprene, polyisobutylene, poly(styrene-butadiene-styrene), the polyurethanes, and silicones.

Polyisoprene, polybutadiene, polychloroprene:

Polyisoprene, polybutadiene, and polychloroprene are all polymerized from diene monomers, and therefore have one double bond per monomer when polymerized. This double bond allows the polymers to be converted to elastomers by vulcanization (essentially, sulfur is used to form crosslinks between the double bonds by heating). This would easily allow homogeneous multilayer soft lithography by incomplete vulcanization of the layers to be bonded; photoresist encapsulation would be possible by a similar mechanism.

Polyisobutylene:

Pure polyisobutylene has no double bonds, but is crosslinked to use as an elastomer by including a small amount (~1%) of isoprene in the polymerization.

The isoprene monomers give pendant double bonds on the polyisobutylene backbone, which may then be vulcanized as above.

Poly(styrene-butadiene-styrene):

Poly(styrene-butadiene-styrene) is produced by living anionic polymerization (that is, there is no natural chain-terminating step in the reaction), so "live" polymer ends can exist in the cured polymer. This makes it a natural candidate for the present photoresist encapsulation system (where there will be plenty of unreacted monomer in the liquid layer poured on top of the cured layer). Incomplete curing would allow homogeneous multilayer soft lithography (A to A bonding). The chemistry also facilitates making one layer with extra butadiene ("A") and coupling agent and the other layer ("B") with a butadiene deficit (for *hetero*geneous multilayer soft lithography). SBS is a "thermoset elastomer", meaning that above a certain temperature it melts and becomes plastic (as opposed to elastic); reducing the temperature yields the elastomer again. Thus, layers can be bonded together by heating.

Polyurethanes:

Polyurethanes are produced from di-isocyanates (A-A) and di-alcohols or di-amines (B-B); since there are a large variety of di-isocyanates and di-alcohols/amines, the number of different types of polyurethanes is huge. The A vs. B nature of the polymers, however, would make them useful for heterogeneous multilayer soft lithography just as RTV 615 is: by using excess A-A in one layer and excess B-B in the other layer.

Silicones:

Silicone polymers probably have the greatest structural variety, and almost certainly have the greatest number of commercially available formulations. The vinyl-to-(Si-H) crosslinking of RTV 615 (which allows both heterogeneous multilayer soft lithography and photoresist encapsulation) has already been discussed, but this is only one of several crosslinking methods used in silicone polymer chemistry.

5. Operation of Device

Figs. 7B and 7H together show the closing of a first flow channel by pressurizing a second flow channel, with Fig. 7B (a front sectional view cutting through flow channel 32 in corresponding Fig. 7A), showing an open first flow channel 30; with Fig. 7H showing first flow channel 30 closed by pressurization of the second flow channel 32.

Referring to Fig. 7B, first flow channel 30 and second flow channel 32 are shown. Membrane 25 separates the flow channels, forming the top of first flow channel 30 and the bottom of second flow channel 32. As can be seen, flow channel 30 is "open".

As can be seen in Fig. 7H, pressurization of flow channel 32 (either by gas or liquid introduced therein) causes membrane 25 to deflect downward, thereby pinching off flow F passing through flow channel 30. Accordingly, by varying the pressure in channel 32, a linearly actuable valving system is provided such that flow channel 30 can be opened or closed by moving membrane 25 as desired. (For illustration purposes only, channel 30 in Fig. 7G is shown in a "mostly closed" position, rather than a "fully closed" position).

Since such valves are actuated by moving the roof of the channels themselves (i.e.: moving membrane 25) valves and pumps produced by this technique have a truly zero dead volume, and switching valves made by this technique have a dead volume approximately equal to the active volume of the valve, for example about 100 x 100 x 10 μm = 100pL. Such dead volumes and areas consumed by the moving membrane are approximately two orders of magnitude smaller than known conventional microvalves. Smaller and larger valves and switching valves are contemplated in the present invention, and a non-exclusive list of ranges of dead volume includes 1 aL to 1 uL, 100 aL to 100 nL, 1 fL to 10 nL, 100 fL to 1 nL, and 1 pL to 100 pL

The extremely small volumes capable of being delivered by pumps and valves in accordance with the present invention represent a substantial advantage. Specifically, the smallest known volumes of fluid capable of being manually metered is around 0.1 μl. The smallest known volumes capable of being metered by automated systems is about ten-times larger (1 μl). Utilizing pumps and valves in accordance with the present invention, volumes of liquid of 10 nl or smaller can routinely be metered and dispensed. The accurate metering of extremely small volumes of fluid enabled by the

present invention would be extremely valuable in a large number of biological applications, including diagnostic tests and assays.

Equation 1 represents a highly simplified mathematical model of deflection of a rectangular, linear, elastic, isotropic plate of uniform thickness by an applied pressure:

(1) $w = (BPb^4)/(Eh^3)$, where:

w = deflection of plate;
B = shape coefficient (dependent upon length vs. width and support of edges of plate);
P = applied pressure;
b = plate width
E = Young's modulus; and
h = plate thickness.

Thus even in this extremely simplified expression, deflection of an elastomeric membrane in response to a pressure will be a function of: the length, width, and thickness of the membrane, the flexibility of the membrane (Young's modulus), and the applied actuation force. Because each of these parameters will vary widely depending upon the actual dimensions and physical composition of a particular elastomeric device in accordance with the present invention, a wide range of membrane thicknesses and elasticities, channel widths, and actuation forces are contemplated by the present invention.

It should be understood that the formula just presented is only an approximation, since in general the membrane does not have uniform thickness, the membrane thickness is not necessarily small compared to the length and width, and the deflection is not necessarily small compared to length, width, or thickness of the membrane. Nevertheless, the equation serves as a useful guide for adjusting variable parameters to achieve a desired response of deflection versus applied force.

Figs. 8A and 8B illustrate valve opening vs. applied pressure for a 100 μm wide first flow channel 30 and a 50 μm wide second flow channel 32. The membrane of this device was formed by a layer of General Electric Silicones RTV 615 having a thickness of approximately 30μm and a Young's modulus of approximately 750 kPa. Figs. 21a and 21b show the extent of opening of the valve to be substantially linear over most of the range of applied pressures.

Air pressure was applied to actuate the membrane of the device through a 10 cm long piece of plastic tubing having an outer diameter of 0.025" connected to a 25 mm piece of stainless steel hypodermic tubing with an outer diameter of 0.025" and an inner diameter of 0.013". This tubing was placed into contact with the control channel by insertion into the elastomeric block in a direction normal to the control channel. Air pressure was applied to the hypodermic tubing from an external LHDA miniature solenoid valve manufactured by Lee Co.

In certain embodiments, it may be useful to apply both positive and negative pressures to actuate the membrane. For example, where the elastomer material is relatively inflexible, an extremely rapid response time is desired, or the flow channel dimensions are small, it may be useful to apply a positive pressure to a control channel actuate the membrane into the flow channel, followed by a negative pressure to cause the membrane to be displaced out of the flow channel.

Moreover, it may also be useful to cause movement of fluid through the microfluidic device by the direct application of pressure to the flow channel, such as the application of positive pressure directly to the flow channel inlet, or application of negative pressure directly to the flow channel outlet. Direct application of pressure alone can drive the flow of fluid within the microfluidic device, or direct pressure may be utilized in conjunction with actuation of membranes overlying the flow channel. Pressure applied directly to the flow channel can also serve to alter the speed of movement of materials thought the flow channel as desired.

While control of the flow of material through the device has so far been described utilizing applied gas pressure, other fluids could be used. For example, air is compressible, and thus experiences some finite delay between the time of application of pressure by the external solenoid valve and the time that this pressure is experienced by the membrane. In an alternative embodiment of the present invention, pressure could be applied from an external source to a noncompressible fluid such as water or hydraulic oils, resulting in a near-instantaneous transfer of applied pressure to the membrane. However, if the displaced volume of the valve is large or the control channel is narrow, higher viscosity of a control fluid may contribute to delay in actuation. The optimal medium for transferring pressure will therefore depend upon the particular application and device configuration, and both gaseous and liquid media are contemplated by the invention.

While external applied pressure as described above has been applied by a pump/tank system through a pressure regulator and external miniature valve, other methods of applying external pressure are also contemplated in the present invention, including gas tanks, compressors, piston systems, and columns of liquid. Also contemplated is the use of naturally occurring pressure sources such as may be found inside living organisms, such as blood pressure, gastric pressure, the pressure present in the cerebro-spinal fluid, pressure present in the intra-ocular space, and the pressure exerted by muscles during normal flexure. Other methods of regulating external pressure are also contemplated, such as miniature valves, pumps, macroscopic peristaltic pumps, pinch valves, and other types of fluid regulating equipment such as is known in the art.

As can be seen, the response of valves in accordance with embodiments of the present invention have been experimentally shown to be almost perfectly linear over a large portion of its range of travel, with minimal hysteresis. Accordingly, the present valves are ideally suited for microfluidic metering and fluid control. The linearity of the valve response demonstrates that the individual valves are well modeled as Hooke's Law springs. Furthermore, high pressures in the flow channel (i.e.: back pressure) can be countered simply by increasing the actuation pressure. Experimentally, the present inventors have achieved valve closure at back pressures of 70 kPa, but higher pressures are also contemplated. The following is a nonexclusive list of pressure ranges encompassed by the present invention: 10 Pa - 25 MPa; 100 Pa - 10 Mpa, 1 kPa - 1 MPa, 1 kPa - 300 kPa, 5 kPa-200 kPa, and 15 kPa - 100 kPa.

While valves and pumps do not require linear actuation to open and close, linear response does allow valves to more easily be used as metering devices. In one embodiment of the invention, the opening of the valve is used to control flow rate by being partially actuated to a known degree of closure. Linear valve actuation makes it easier to determine the amount of actuation force required to close the valve to a desired degree of closure. Another benefit of linear actuation is that the force required for valve actuation may be easily determined from the pressure in the flow channel. If actuation is linear, increased pressure in the flow channel may be countered by adding the same pressure (force per unit area) to the actuated portion of the valve.

Linearity of a valve depends on the structure, composition, and method of actuation of the valve structure. Furthermore, whether linearity is a desirable characteristic in a valve depends on the application. Therefore, both linearly and non-

linearly actuable valves are contemplated in the present invention, and the pressure ranges over which a valve is linearly actuable will vary with the specific embodiment.

Fig. 9 illustrates time response (i.e.: closure of valve as a function of time in response to a change in applied pressure) of a 100μm×100μm×10μm RTV microvalve with 10-cm-long air tubing connected from the chip to a pneumatic valve as described above.

Two periods of digital control signal, actual air pressure at the end of the tubing and valve opening are shown in Fig. 9. The pressure applied on the control line is 100 kPa, which is substantially higher than the ~40 kPa required to close the valve. Thus, when closing, the valve is pushed closed with a pressure 60 kPa greater than required. When opening, however, the valve is driven back to its rest position only by its own spring force (≤ 40 kPa). Thus, τ_{close} is expected to be smaller than τ_{open}. There is also a lag between the control signal and control pressure response, due to the limitations of the miniature valve used to control the pressure. Calling such lags t and the 1/e time constants τ, the values are: t_{open} = 3.63 ms, τ_{open} = 1.88 ms, t_{close} = 2.15 ms, τ_{close} = 0.51 ms. If 3τ each are allowed for opening and closing, the valve runs comfortably at 75 Hz when filled with aqueous solution.

If one used another actuation method which did not suffer from opening and closing lag, this valve would run at ~375 Hz. Note also that the spring constant can be adjusted by changing the membrane thickness; this allows optimization for either fast opening or fast closing. The spring constant could also be adjusted by changing the elasticity (Young's modulus) of the membrane, as is possible by introducing dopant into the membrane or by utilizing a different elastomeric material to serve as the membrane (described above in conjunction with Figs. 7C-7H.)

When experimentally measuring the valve properties as illustrated in Fig. 9 the valve opening was measured by fluorescence. In these experiments, the flow channel was filled with a solution of fluorescein isothiocyanate (FITC) in buffer (pH ≥ 8) and the fluorescence of a square area occupying the center ~1/3rd of the channel is monitored on an epi-fluorescence microscope with a photomultiplier tube with a 10 kHz bandwidth. The pressure was monitored with a Wheatstone-bridge pressure sensor (SenSym SCC15GD2) pressurized simultaneously with the control line through nearly identical pneumatic connections.

6. Flow Channel Cross Sections

The flow channels of the present invention may optionally be designed with different cross sectional sizes and shapes, offering different advantages, depending upon their desired application. For example, the cross sectional shape of the lower flow channel may have a curved upper surface, either along its entire length or in the region disposed under an upper cross channel). Such a curved upper surface facilitates valve sealing, as follows.

Referring to Fig. 10, a cross sectional view (similar to that of Fig. 7B) through flow channels 30 and 32 is shown. As can be seen, flow channel 30 is rectangular in cross sectional shape. In an alternate preferred aspect of the invention, as shown in Fig. 20, the cross-section of a flow channel 30 instead has an upper curved surface.

Referring first to Fig. 10, when flow channel 32 is pressurized, the membrane portion 25 of elastomeric block 24 separating flow channels 30 and 32 will move downwardly to the successive positions shown by the dotted lines 25A, 25B, 25C, 25D, and 25E. As can be seen, incomplete sealing may possibly result at the edges of flow channel 30 adjacent planar substrate 14.

In the alternate preferred embodiment of Fig. 11, flow channel 30a has a curved upper wall 25A. When flow channel 32 is pressurized, membrane portion 25 will move downwardly to the successive positions shown by dotted lines 25A2, 25A3, 25A4 and 25A5, with edge portions of the membrane moving first into the flow channel, followed by top membrane portions. An advantage of having such a curved upper surface at membrane 25A is that a more complete seal will be provided when flow channel 32 is pressurized. Specifically, the upper wall of the flow channel 30 will provide a continuous contacting edge against planar substrate 14, thereby avoiding the "island" of contact seen between wall 25 and the bottom of flow channel 30 in Fig. 10.

Another advantage of having a curved upper flow channel surface at membrane 25A is that the membrane can more readily conform to the shape and volume of the flow channel in response to actuation. Specifically, where a rectangular flow channel is employed, the entire perimeter (2x flow channel height, plus the flow channel width) must be forced into the flow channel. However where an arched flow channel is used, a smaller perimeter of material (only the semi-circular arched portion) must be forced into the channel. In this manner, the membrane requires less change in perimeter for actuation and is therefore more responsive to an applied actuation force to block the flow channel

In an alternate aspect, (not illustrated), the bottom of flow channel 30 is rounded such that its curved surface mates with the curved upper wall 25A as seen in Fig. 20 described above.

In summary, the actual conformational change experienced by the membrane upon actuation will depend upon the configuration of the particular elastomeric structure. Specifically, the conformational change will depend upon the length, width, and thickness profile of the membrane, its attachment to the remainder of the structure, and the height, width, and shape of the flow and control channels and the material properties of the elastomer used. The conformational change may also depend upon the method of actuation, as actuation of the membrane in response to an applied pressure will vary somewhat from actuation in response to a magnetic or electrostatic force.

Moreover, the desired conformational change in the membrane will also vary depending upon the particular application for the elastomeric structure. In the simplest embodiments described above, the valve may either be open or closed, with metering to control the degree of closure of the valve. In other embodiments however, it may be desirable to alter the shape of the membrane and/or the flow channel in order to achieve more complex flow regulation. For instance, the flow channel could be provided with raised protrusions beneath the membrane portion, such that upon actuation the membrane shuts off only a percentage of the flow through the flow channel, with the percentage of flow blocked insensitive to the applied actuation force.

Many membrane thickness profiles and flow channel cross-sections are contemplated by the present invention, including rectangular, trapezoidal, circular, ellipsoidal, parabolic, hyperbolic, and polygonal, as well as sections of the above shapes. More complex cross-sectional shapes, such as the embodiment with protrusions discussed immediately above or an embodiment having concavities in the flow channel, are also contemplated by the present invention.

In addition, while the invention is described primarily above in conjunction with an embodiment wherein the walls and ceiling of the flow channel are formed from elastomer, and the floor of the channel is formed from an underlying substrate, the present invention is not limited to this particular orientation. Walls and floors of channels could also be formed in the underlying substrate, with only the ceiling of the flow channel constructed from elastomer. This elastomer flow channel ceiling would project downward into the channel in response to an applied actuation force,

thereby controlling the flow of material through the flow channel. In general, monolithic elastomer structures as described elsewhere in the instant application are preferred for microfluidic applications. However, it may be useful to employ channels formed in the substrate where such an arrangement provides advantages. For instance, a substrate including optical waveguides could be constructed so that the optical waveguides direct light specifically to the side of a microfluidic channel.

7. Alternate Valve Actuation Techniques

In addition to pressure based actuation systems described above, optional electrostatic and magnetic actuation systems are also contemplated, as follows.

Electrostatic actuation can be accomplished by forming oppositely charged electrodes (which will tend to attract one another when a voltage differential is applied to them) directly into the monolithic elastomeric structure. For example, referring to Fig. 7B, an optional first electrode 70 (shown in phantom) can be positioned on (or in) membrane 25 and an optional second electrode 72 (also shown in phantom) can be positioned on (or in) planar substrate 14. When electrodes 70 and 72 are charged with opposite polarities, an attractive force between the two electrodes will cause membrane 25 to deflect downwardly, thereby closing the "valve" (i.e.: closing flow channel 30).

For the membrane electrode to be sufficiently conductive to support electrostatic actuation, but not so mechanically stiff so as to impede the valve's motion, a sufficiently flexible electrode must be provided in or over membrane 25. Such an electrode may be provided by a thin metallization layer, doping the polymer with conductive material, or making the surface layer out of a conductive material.

In an exemplary aspect, the electrode present at the deflecting membrane can be provided by a thin metallization layer which can be provided, for example, by sputtering a thin layer of metal such as 20 nm of gold. In addition to the formation of a metallized membrane by sputtering, other metallization approaches such as chemical epitaxy, evaporation, electroplating, and electroless plating are also available. Physical transfer of a metal layer to the surface of the elastomer is also available, for example by evaporating a metal onto a flat substrate to which it adheres poorly, and then placing the elastomer onto the metal and peeling the metal off of the substrate.

A conductive electrode 70 may also be formed by depositing carbon black (i.e. Cabot Vulcan XC72R) on the elastomer surface, either by wiping on the dry powder or by exposing the elastomer to a suspension of carbon black in a solvent which causes

swelling of the elastomer, (such as a chlorinated solvent in the case of PDMS). Alternatively, the electrode 70 may be formed by constructing the entire layer 20 out of elastomer doped with conductive material (i.e. carbon black or finely divided metal particles). Yet further alternatively, the electrode may be formed by electrostatic deposition, or by a chemical reaction that produces carbon. In experiments conducted by the present inventors, conductivity was shown to increase with carbon black concentration from 5.6×10^{-16} to about 5×10^{-3} $(\Omega\text{-cm})^{-1}$. The lower electrode 72, which is not required to move, may be either a compliant electrode as described above, or a conventional electrode such as evaporated gold, a metal plate, or a doped semiconductor electrode.

Magnetic actuation of the flow channels can be achieved by fabricating the membrane separating the flow channels with a magnetically polarizable material such as iron, or a permanently magnetized material such as polarized NdFeB. In experiments conducted by the present inventors, magnetic silicone was created by the addition of iron powder (about 1 um particle size), up to 20% iron by weight.

Where the membrane is fabricated with a magnetically polarizable material, the membrane can be actuated by attraction in response to an applied magnetic field Where the membrane is fabricated with a material capable of maintaining permanent magnetization, the material can first be magnetized by exposure to a sufficiently high magnetic field, and then actuated either by attraction or repulsion in response to the polarity of an applied inhomogenous magnetic field.

The magnetic field causing actuation of the membrane can be generated in a variety of ways. In one embodiment, the magnetic field is generated by an extremely small inductive coil formed in or proximate to the elastomer membrane. The actuation effect of such a magnetic coil would be localized, allowing actuation of individual pump and/or valve structures. Alternatively, the magnetic field could be generated by a larger, more powerful source, in which case actuation would be global and would actuate multiple pump and/or valve structures at one time.

It is also possible to actuate the device by causing a fluid flow in the control channel based upon the application of thermal energy, either by thermal expansion or by production of gas from liquid. For example, in one alternative embodiment in accordance with the present invention, a pocket of fluid (e.g. in a fluid-filled control channel) is positioned over the flow channel. Fluid in the pocket can be in

communication with a temperature variation system, for example a heater. Thermal expansion of the fluid, or conversion of material from the liquid to the gas phase, could result in an increase in pressure, closing the adjacent flow channel. Subsequent cooling of the fluid would relieve pressure and permit the flow channel to open.

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8. Networked Systems

Figs. 12A and 12B show a views of a single on/off valve, identical to the systems set forth above, (for example in Fig. 7A). Figs. 13A and 13B shows a peristaltic pumping system comprised of a plurality of the single addressable on/off valves as seen 10 in Fig. 12, but networked together. Fig. 14 is a graph showing experimentally achieved pumping rates vs. frequency for the peristaltic pumping system of Fig. 13. Figs. 15A and 15B show a schematic view of a plurality of flow channels which are controllable by a single control line. This system is also comprised of a plurality of the single addressable on/off valves of Fig. 12, multiplexed together, but in a different arrangement than that of 15 Fig. 12. Fig. 16 is a schematic illustration of a multiplexing system adapted to permit fluid flow through selected channels, comprised of a plurality of the single on/off valves of Fig. 12, joined or networked together.

Referring first to Figs. 12A and 12B, a schematic of flow channels 30 and 32 is shown. Flow channel 30 preferably has a fluid (or gas) flow F passing therethrough. 20 Flow channel 32, (which crosses over flow channel 30, as was already explained herein), is pressurized such that membrane 25 separating the flow channels may be depressed into the path of flow channel 30, shutting off the passage of flow F therethrough, as has been explained. As such, "flow channel" 32 can also be referred to as a "control line" which actuates a single valve in flow channel 30. In Figs. 12 to 15, a plurality of such 25 addressable valves are joined or networked together in various arrangements to produce pumps, capable of peristaltic pumping, and other fluidic logic applications.

Referring to Fig. 13A and 13B, a system for peristaltic pumping is provided, as follows. A flow channel 30 has a plurality of generally parallel flow channels (i.e.: control lines) 32A, 32B and 32C passing thereover. By pressurizing 30 control line 32A, flow F through flow channel 30 is shut off under membrane 25A at the intersection of control line 32A and flow channel 30. Similarly, (but not shown), by pressurizing control line 32B, flow F through flow channel 30 is shut off under membrane 25B at the intersection of control line 32B and flow channel 30, etc.

Each of control lines 32A, 32B, and 32C is separately addressable. Therefore, peristalsis may be actuated by the pattern of actuating 32A and 32C together, followed by 32A, followed by 32A and 32B together, followed by 32B, followed by 32B and C together, etc. This corresponds to a successive "101, 100, 110, 010, 011, 001" pattern, where "0" indicates "valve open" and "1" indicates "valve closed." This peristaltic pattern is also known as a 120° pattern (referring to the phase angle of actuation between three valves). Other peristaltic patterns are equally possible, including 60° and 90° patterns.

In experiments performed by the inventors, a pumping rate of 2.35 nL/s was measured by measuring the distance traveled by a column of water in thin (0.5 mm i.d.) tubing; with 100x100x10 μm valves under an actuation pressure of 40 kPa. The pumping rate increased with actuation frequency until approximately 75 Hz, and then was nearly constant until above 200 Hz. The valves and pumps are also quite durable and the elastomer membrane, control channels, or bond have never been observed to fail. In experiments performed by the inventors, none of the valves in the peristaltic pump described herein show any sign of wear or fatigue after more than 4 million actuations. In addition to their durability, they are also gentle. A solution of *E. Coli* pumped through a channel and tested for viability showed a 94% survival rate.

Fig. 14 is a graph showing experimentally achieved pumping rates vs. frequency for the peristaltic pumping system of Fig. 13.

Figs. 15A and 15B illustrates another way of assembling a plurality of the addressable valves of Fig. 12. Specifically, a plurality of parallel flow channels 30A, 30B, and 30C are provided. Flow channel (i.e.: control line) 32 passes thereover across flow channels 30A, 30B, and 30C. Pressurization of control line 32 simultaneously shuts off flows F1, F2 and F3 by depressing membranes 25A, 25B, and 25C located at the intersections of control line 32 and flow channels 30A, 30B, and 30C.

Fig. 16 is a schematic illustration of a multiplexing system adapted to selectively permit fluid to flow through selected channels, as follows. The downward deflection of membranes separating the respective flow channels from a control line passing thereabove (for example, membranes 25A, 25B, and 25C in Figs. 15A and 15B) depends strongly upon the membrane dimensions. Accordingly, by varying the widths of flow channel control line 32 in Figs. 15A and 15B, it is possible to have a control line

pass over multiple flow channels, yet only actuate (i.e.: seal) desired flow channels. Fig. 16 illustrates a schematic of such a system, as follows.

A plurality of parallel flow channels 30A, 30B, 30C, 30D, 30E and 30F are positioned under a plurality of parallel control lines 32A, 32B, 32C, 32D, 32E and 32F. Control channels 32A, 32B, 32C, 32D, 32E and 32F are adapted to shut off fluid flows F1, F2, F3, F4, F5 and F6 passing through parallel flow channels 30A, 30B, 30C, 30D, 30E and 30F using any of the valving systems described above, with the following modification.

Each of control lines 32A, 32B, 32C, 32D, 32E and 32F have both wide and narrow portions. For example, control line 32A is wide in locations disposed over flow channels 30A, 30C and 30E. Similarly, control line 32B is wide in locations disposed over flow channels 30B, 30D and 30F, and control line 32C is wide in locations disposed over flow channels 30A, 30B, 30E and 30F.

At the locations where the respective control line is wide, its pressurization will cause the membrane (25) separating the flow channel and the control line to depress significantly into the flow channel, thereby blocking the flow passage therethrough. Conversely, in the locations where the respective control line is narrow, membrane (25) will also be narrow. Accordingly, the same degree of pressurization will not result in membrane (25) becoming depressed into the flow channel (30). Therefore, fluid passage thereunder will not be blocked.

For example, when control line 32A is pressurized, it will block flows F1, F3 and F5 in flow channels 30A, 30C and 30E. Similarly, when control line 32C is pressurized, it will block flows F1, F2, F5 and F6 in flow channels 30A, 30B, 30E and 30F. As can be appreciated, more than one control line can be actuated at the same time. For example, control lines 32A and 32C can be pressurized simultaneously to block all fluid flow except F4 (with 32A blocking F1, F3 and F5; and 32C blocking F1, F2, F5 and F6).

By selectively pressurizing different control lines (32) both together and in various sequences, a great degree of fluid flow control can be achieved. Moreover, by extending the present system to more than six parallel flow channels (30) and more than four parallel control lines (32), and by varying the positioning of the wide and narrow regions of the control lines, very complex fluid flow control systems may be fabricated. A property of such systems is that it is possible to turn on any one flow channel out of n flow channels with only $2(\log_2 n)$ control lines.

9. Selectively Addressable Reaction Chambers Along Flow Lines

In a further embodiment of the invention, illustrated in Figs. 17A, 17B, 17C and 17D, a system for selectively directing fluid flow into one more of a plurality of reaction chambers disposed along a flow line is provided.

Fig. 17A shows a top view of a flow channel 30 having a plurality of reaction chambers 80A and 80B disposed therealong. Preferably flow channel 30 and reaction chambers 80A and 80B are formed together as recesses into the bottom surface of a first layer 100 of elastomer.

Fig. 17B shows a bottom plan view of another elastomeric layer 110 with two control lines 32A and 32B each being generally narrow, but having wide extending portions 33A and 33B formed as recesses therein.

As seen in the exploded view of Fig. 17C, and assembled view of Fig. 17D, elastomeric layer 110 is placed over elastomeric layer 100. Layers 100 and 110 are then bonded together, and the integrated system operates to selectively direct fluid flow F (through flow channel 30) into either or both of reaction chambers 80A and 80B, as follows. Pressurization of control line 32A will cause the membrane 25 (i.e.: the thin portion of elastomer layer 100 located below extending portion 33A and over regions 82A of reaction chamber 80A) to become depressed, thereby shutting off fluid flow passage in regions 82A, effectively sealing reaction chamber 80 from flow channel 30. As can also be seen, extending portion 33A is wider than the remainder of control line 32A. As such, pressurization of control line 32A will not result in control line 32A sealing flow channel 30.

As can be appreciated, either or both of control lines 32A and 32B can be actuated at once. When both control lines 32A and 32B are pressurized together, sample flow in flow channel 30 will enter neither of reaction chambers 80A or 80B.

The concept of selectably controlling fluid introduction into various addressable reaction chambers disposed along a flow line (Figs. 17A-D) can be combined with concept of selectably controlling fluid flow through one or more of a plurality of parallel flow lines (Fig. 16) to yield a system in which a fluid sample or samples can be can be sent to any particular reaction chamber in an array of reaction chambers. An example of such a system is provided in Fig. 18, in which parallel control channels 32A, 32B and 32C with extending portions 34 (all shown in phantom) selectively direct fluid flows F1 and F2 into any of the array of reaction wells 80A, 80B, 80C or 80D as

explained above; while pressurization of control lines 32C and 32D selectively shuts off flows F2 and F1, respectively.

In yet another novel embodiment, fluid passage between parallel flow channels is possible. Referring to Fig. 19, either or both of control lines 32A or 32D can be depressurized such that fluid flow through lateral passageways 35 (between parallel flow channels 30A and 30B) is permitted. In this aspect of the invention, pressurization of control lines 32C and 32D would shut flow channel 30A between 35A and 35B, and would also shut lateral passageways 35B. As such, flow entering as flow F1 would sequentially travel through 30A, 35A and leave 30B as flow F4.

10. Switchable Flow Arrays

In yet another novel embodiment, fluid passage can be selectively directed to flow in either of two perpendicular directions. An example of such a "switchable flow array" system is provided in Figs. 20A to 20D. Fig. 20A shows a bottom view of a first layer of elastomer 90, (or any other suitable substrate), having a bottom surface with a pattern of recesses forming a flow channel grid defined by an array of solid posts 92, each having flow channels passing therearound.

In preferred aspects, an additional layer of elastomer is bound to the top surface of layer 90 such that fluid flow can be selectively directed to move either in direction F1, or perpendicular direction F2. Fig. 20 is a bottom view of the bottom surface of the second layer of elastomer 95 showing recesses formed in the shape of alternating "vertical" control lines 96 and "horizontal" control lines 94. "Vertical" control lines 96 have the same width therealong, whereas "horizontal" control lines 94 have alternating wide and narrow portions, as shown.

Elastomeric layer 95 is positioned over top of elastomeric layer 90 such that "vertical" control lines 96 are positioned over posts 92 as shown in Fig. 20C and "horizontal" control lines 94 are positioned with their wide portions between posts 92, as shown in Fig. 20D.

As can be seen in Fig. 20C, when "vertical" control lines 96 are pressurized, the membrane of the integrated structure formed by the elastomeric layer initially positioned between layers 90 and 95 in regions 98 will be deflected downwardly over the array of flow channels such that flow in only able to pass in flow direction F2 (i.e.: vertically), as shown.

As can be seen in Fig. 20D, when "horizontal" control lines 94 are pressurized, the membrane of the integrated structure formed by the elastomeric layer initially positioned between layers 90 and 95 in regions 99 will be deflected downwardly over the array of flow channels, (but only in the regions where they are widest), such that flow in only able to pass in flow direction F1 (i.e.: horizontally), as shown.

The design illustrated in Figs. 20 allows a switchable flow array to be constructed from only two elastomeric layers, with no vertical vias passing between control lines in different elastomeric layers required. If all vertical flow control lines 94 are connected, they may be pressurized from one input. The same is true for all horizontal flow control lines 96.

11. Normally-Closed Valve Structure

Figs. 7B and 7H above depict a valve structure in which the elastomeric membrane is moveable from a first relaxed position to a second actuated position in which the flow channel is blocked. However, the present invention is not limited to this particular valve configuration.

Figs. 21A-21J show a variety of views of a normally-closed valve structure in which the elastomeric membrane is moveable from a first relaxed position blocking a flow channel, to a second actuated position in which the flow channel is open, utilizing a negative control pressure.

Fig. 21A shows a plan view, and Fig. 21B shows a cross sectional view along line 42B-42B', of normally-closed valve 4200 in an unactuated state. Flow channel 4202 and control channel 4204 are formed in elastomeric block 4206 overlying substrate 4205. Flow channel 4202 includes a first portion 4202a and a second portion 4202b separated by separating portion 4208. Control channel 4204 overlies separating portion 4208. As shown in Fig. 42B, in its relaxed, unactuated position, separating portion 4008 remains positioned between flow channel portions 4202a and 4202b, interrupting flow channel 4202.

Fig. 21C shows a cross-sectional view of valve 4200 wherein separating portion 4208 is in an actuated position. When the pressure within control channel 4204 is reduced to below the pressure in the flow channel (for example by vacuum pump), separating portion 4208 experiences an actuating force drawing it into control channel 4204. As a result of this actuation force membrane 4208 projects into control channel 4204, thereby removing the obstacle to a flow of material through flow channel 4202 and

creating a passageway 4203. Upon elevation of pressure within control channel 4204, separating portion 4208 will assume its natural position, relaxing back into and obstructing flow channel 4202.

The behavior of the membrane in response to an actuation force may be changed by varying the width of the overlying control channel. Accordingly, Figs. 21D-42H show plan and cross-sectional views of an alternative embodiment of a normally-closed valve 4201 in which control channel 4207 is substantially wider than separating portion 4208. As shown in cross-sectional views Fig. 21E-F along line 42E-42E' of Fig. 21D, because a larger area of elastomeric material is required to be moved during actuation, the actuation force necessary to be applied is reduced.

Figs. 21G and H show a cross-sectional views along line 40G-40G' of Fig. 21D. In comparison with the unactuated valve configuration shown in Fig. 21G, Fig. 21H shows that reduced pressure within wider control channel 4207 may under certain circumstances have the unwanted effect of pulling underlying elastomer 4206 away from substrate 4205, thereby creating undesirable void 4212.

Accordingly, Fig. 21I shows a plan view, and Fig. 21J shows a cross-sectional view along line 21J-21J' of Fig. 21I, of valve structure 4220 which avoids this problem by featuring control line 4204 with a minimum width except in segment 4204a overlapping separating portion 4208. As shown in Fig. 21J, even under actuated conditions the narrower cross-section of control channel 4204 reduces the attractive force on the underlying elastomer material 4206, thereby preventing this elastomer material from being drawn away from substrate 4205 and creating an undesirable void.

While a normally-closed valve structure actuated in response to pressure is shown in Figs. 21A-21J, a normally-closed valve in accordance with the present invention is not limited to this configuration. For example, the separating portion obstructing the flow channel could alternatively be manipulated by electric or magnetic fields, as described extensively above.

12. Side-Actuated Valve

While the above description has focused upon microfabricated elastomeric valve structures in which a control channel is positioned above and separated by an intervening elastomeric membrane from an underlying flow channel, the present invention is not limited to this configuration. Figs. 22A and 22B show plan views of one

embodiment of a side-actuated valve structure in accordance with one embodiment of the present invention.

Fig. 22A shows side-actuated valve structure 4800 in an unactuated position. Flow channel 4802 is formed in elastomeric layer 4804. Control channel 4806 abutting flow channel 4802 is also formed in elastomeric layer 4804. Control channel 4806 is separated from flow channel 4802 by elastomeric membrane portion 4808. A second elastomeric layer (not shown) is bonded over bottom elastomeric layer 4804 to enclose flow channel 4802 and control channel 4806.

Fig. 22B shows side-actuated valve structure 4800 in an actuated position. In response to a build up of pressure within control channel 4806, membrane 4808 deforms into flow channel 4802, blocking flow channel 4802. Upon release of pressure within control channel 4806, membrane 4808 would relax back into control channel 4806 and open flow channel 4802.

While a side-actuated valve structure actuated in response to pressure is shown in Figs. 22A and 22B, a side-actuated valve in accordance with the present invention is not limited to this configuration. For example, the elastomeric membrane portion located between the abutting flow and control channels could alternatively be manipulated by electric or magnetic fields, as described extensively above.

13. Composite Structures

Microfabricated elastomeric structures of the present invention may be combined with non-elastomeric materials to create composite structures. Figure 23 shows a cross-sectional view of one embodiment of a composite structure in accordance with the present invention. Figure 23 shows composite valve structure 5700 including first, thin elastomer layer 5702 overlying semiconductor-type substrate 5704 having channel 5706 formed therein. Second, thicker elastomer layer 5708 overlies first elastomer layer 5702. Actuation of first elastomer layer 5702 to drive it into channel 5706, will cause composite structure 5700 to operate as a valve.

Figure 24 shows a cross-sectional view of a variation on this theme, wherein thin elastomer layer 5802 is sandwiched between two hard, semiconductor substrates 5804 and 5806, with lower substrate 5804 featuring channel 5808. Again, actuation of thin elastomer layer 5802 to drive it into channel 5808 will cause composite structure 5810 to operate as a valve.

The structures shown in Figures 23 or 24 may be fabricated utilizing either the multilayer soft lithography or encapsulation techniques described above. In the multilayer soft lithography method, the elastomer layer(s) would be formed and then placed over the semiconductor substrate bearing the channel. In the encapsulation method, the channel would be first formed in the semiconductor substrate, and then the channel would be filled with a sacrificial material such as photoresist. The elastomer would then be formed in place over the substrate, with removal of the sacrificial material producing the channel overlaid by the elastomer membrane. As is discussed in detail below in connection with bonding of elastomer to other types of materials, the encapsulation approach may result in a stronger seal between the elastomer membrane component and the underlying nonelastomer substrate component.

As shown in Figures 23 and 24, a composite structure in accordance with embodiments of the present invention may include a hard substrate that bears a passive feature such as a channels. However, the present invention is not limited to this approach, and the underlying hard substrate may bear active features that interact with an elastomer component bearing a recess. This is shown in Figure 25, wherein composite structure 5900 includes elastomer component 5902 containing recess 5904 having walls 5906 and ceiling 5908. Ceiling 5908 forms flexible membrane portion 5909. Elastomer component 5902 is sealed against substantially planar nonelastomeric component 5910 that includes active device 5912. Active device 5912 may interact with material present in recess 5904 and/or flexible membrane portion 5909.

Many types of active structures may be present in the nonelastomer substrate. Active structures that could be present in an underlying hard substrate include, but are not limited to, resistors, capacitors, photodiodes, transistors, chemical field effect transistors (chem FET's), amperometric/coulometric electrochemical sensors, fiber optics, fiber optic interconnects, light emitting diodes, laser diodes, vertical cavity surface emitting lasers (VCSEL's), micromirrors, accelerometers, pressure sensors, flow sensors, CMOS imaging arrays, CCD cameras, electronic logic, microprocessors, thermistors, Peltier coolers, waveguides, resistive heaters, chemical sensors, strain gauges, inductors, actuators (including electrostatic, magnetic, electromagnetic, bimetallic, piezoelectric, shape-memory-alloy based, and others), coils, magnets, electromagnets, magnetic sensors (such as those used in hard drives, superconducting quantum interference devices (SQUIDS) and other types), radio frequency sources and receivers, microwave frequency

sources and receivers, sources and receivers for other regions of the electromagnetic spectrum, radioactive particle counters, and electrometers.

As is well known in the art, a vast variety of technologies can be utilized to fabricate active features in semiconductor and other types of hard substrates, including but not limited printed circuit board (PCB) technology, CMOS, surface micromachining, bulk micromachining, printable polymer electronics, and TFT and other amorphous/polycrystalline techniques as are employed to fabricate laptop and flat screen displays.

A variety of approaches can be employed to seal the elastomeric structure against the nonelastomeric substrate, ranging from the creation of a Van der Waals bond between the elastomeric and nonelastomeric components, to creation of covalent or ionic bonds between the elastomeric and nonelastomeric components of the composite structure. Example approaches to sealing the components together are discussed below, approximately in order of increasing strength.

A first approach is to rely upon the simple hermetic seal resulting from Van der Waals bonds formed when a substantially planar elastomer layer is placed into contact with a substantially planar layer of a harder, non-elastomer material. In one embodiment, bonding of RTV elastomer to a glass substrate created a composite structure capable of withstanding up to about 3-4 psi of pressure. This may be sufficient for many potential applications.

A second approach is to utilize a liquid layer to assist in bonding. One example of this involves bonding elastomer to a hard glass substrate, wherein a weakly acidic solution (5 μl HCl in H_20, pH 2) was applied to a glass substrate. The elastomer component was then placed into contact with the glass substrate, and the composite structure baked at 37°C to remove the water. This resulted in a bond between elastomer and non-elastomer able to withstand a pressure of about 20 psi. In this case, the acid may neutralize silanol groups present on the glass surface, permitting the elastomer and non-elastomer to enter into good Van der Waals contact with each other.

Exposure to ethanol can also cause device components to adhere together. In one embodiment, an RTV elastomer material and a glass substrate were washed with ethanol and then dried under Nitrogen. The RTV elastomer was then placed into contact with the glass and the combination baked for 3 hours at 80°C. Optionally, the RTV may also be exposed to a vacuum to remove any air bubbles trapped between the slide and the

RTV. The strength of the adhesion between elastomer and glass using this method has withstood pressures in excess of 35 psi. The adhesion created using this method is not permanent, and the elastomer may be peeled off of the glass, washed, and resealed against the glass. This ethanol washing approach can also be employed used to cause successive layers of elastomer to bond together with sufficient strength to resist a pressure of 30 psi. In alternative embodiments, chemicals such as other alcohols or diols could be used to promote adhesion between layers.

An embodiment of a method of promoting adhesion between layers of a microfabricated structure in accordance with the present invention comprises exposing a surface of a first component layer to a chemical, exposing a surface of a second component layer to the chemical, and placing the surface of the first component layer into contact with the surface of the second elastomer layer.

A third approach is to create a covalent chemical bond between the elastomer component and functional groups introduced onto the surface of a nonelastomer component. Examples of derivitization of a nonelastomer substrate surface to produce such functional groups include exposing a glass substrate to agents such as vinyl silane or aminopropyltriethoxy silane (APTES), which may be useful to allow bonding of the glass to silicone elastomer and polyurethane elastomer materials, respectively.

A fourth approach is to create a covalent chemical bond between the elastomer component and a functional group native to the surface of the nonelastomer component. For example, RTV elastomer can be created with an excess of vinyl groups on its surface. These vinyl groups can be caused to react with corresponding functional groups present on the exterior of a hard substrate material, for example the Si-H bonds prevalent on the surface of a single crystal silicon substrate after removal of native oxide by etching. In this example, the strength of the bond created between the elastomer component and the nonelastomer component has been observed to exceed the materials strength of the elastomer components.

II. Damper Structure

Embodiments of apparatuses and methods in accordance with the present invention are directed to microfluidic devices comprising pumps, valves, and fluid oscillation dampers. In this respect, the microfluidic devices of the present invention is similar to that described in Unger et al. *Science*, 2000, *288*, 113-116, which is

incorporated herein by reference in its entirety. However, microfluidic devices of the present invention may further comprise a damper.

The advantages of microfluidic devices of the present invention include reduced fluid oscillation within a flow channel which reduces potential variability in detection means. As the fluid is pushed through the flow channel by the pumps, there is a tendency for the fluid to oscillate, i.e., the fluid is pushed through the flow channel in a sinusoidal wave-like fashion. As this oscillating fluid passes through a detector region, different fluid depth passes through the detector region. And depending on a particular detection means used, this difference in fluid depth can cause a higher "background noise" or an inaccurate reading by the detector. By reducing or eliminating this fluid oscillation, the "background noise" is reduced and a more accurate reading by the detector can be achieved.

Preferred devices are constructed by single and multilayer soft lithography (MLSL) as detailed in commonly assigned U.S. Patent Serial Application No. 09/605,520, filed June 27, 2000, which is incorporated herein by reference in its entirety.

Microfluidic devices of the present invention comprise an integrated pump which can be electronic, magnetic, mechanical, or preferably pneumatic pumps. By using a pneumatic pump, microfluidic devices of the present invention allow more precise control of the fluid flow within the fluid channel. In addition, unlike electro-osmotic driven fluid flow, pneumatic pump allows the flow of fluids in both directions, thereby allowing reversible sorting of materials, as discussed in greater detail below. Furthermore, a pneumatic pump provides at least 10 times, preferably at least about 20 times, and more preferably at least about 30 times the fluid flow rate capacity compared to the capacity of electro-osmotic fluid flow.

In addition, microfluidic devices of the present invention may comprise a damper which reduces or eliminates the fluid oscillation within the fluid channel. The damper can any device which attenuates the fluid oscillation. For example, the damper can simply be a channel which is open to the ambient atmosphere and has a thin elastic membrane between the channel and the fluid flow channel. Preferably, the damper is an encapsulated pocket of fluid medium with a thin elastic membrane above the fluid flow channel. The fluid medium can be a liquid or, preferably, a gas. The damper is generally located above the fluid flow channels with a thin membrane, preferably an elastic membrane, between the fluid flow channel and the damper. Typically, there is at least 1 damper posterior to the pump in the direction of the fluid flow. Preferably, there is at

least 2 dampers, more preferably at least 3 dampers and most preferably at least about 5 dampers posterior to the pump.

The width of damper is at least as wide as the width of flow channel that is located below the damper. In this manner, the entire cross-section of the flow channel is covered by the damper to ensure attenuation of fluid oscillation across the entire width of the flow channel. Preferably, the width of damper is at least about 1.1 times the width of flow channel, more preferably at least about 1.3 times the width of the flow channel, and most preferably at least about 1.5 times the width of the flow channel. In this manner, the need for a precise alignment of the damper on top of the flow channel is eliminated.

The damper is separated from the fluid flow channel by a thin membrane. Preferably this thin membrane has sufficient elasticity to deflect "upward" when a fluid having a peak of sinusoidal wave-like passes underneath. In this manner, some of the fluid oscillation energy is absorbed by the damper, thereby reducing the height (i.e., peak) of fluid oscillation. Typically, the thickness of the membrane between the damper and the fluid flow channel depends on a variety of factors including the depth and width of the flow channel, the amount of fluid oscillation produced by the pump and the elasticity (i.e., the material) of the membrane. One of ordinary skill in the art can readily determine the proper membrane thickness to achieve a desired attenuation of fluid oscillation depending on a desired application and materials used.

In general, damper structures in accordance with embodiments of the present invention feature an energy absorber adjacent to the flow channel, the energy absorber configured to experience a physical change in response to an oscillation within the flow channel. As described below, the energy absorber can take several forms, including but not limited to a flexible membrane, a pocket filled with a compressible fluid, and/or flexible walls of the flow channel itself.

Figure 26 shows a cross-sectional view of a first embodiment of a damper structure in accordance with the present invention. Damper structure 2600 comprises cavity 2602 separated from underlying flow channel 2604 by membrane 2606 of elastomer layer 2608 in which cavity 2602 is formed. Oscillation in pressure 2610 in the fluid flowing through underlying flow channel 2604 causes membrane 2606 to flex up and down, thereby absorbing some of the energy of oscillation and providing for a more uniform flow of material through channel 2604. The degree to which damper structure 2600 is capable of absorbing oscillation energy is dictated by a number of factors, including but not limited to, the length of the flow channel covered by the membrane, the

elasticity of the membrane, and the compressability of any liquid material present in the cavity. The longer the membrane and the greater its flexion, the larger amount of oscillation energy that can be absorbed. Moreover, as stated above, the damping effect can be amplified by positioning a series of damper structures along the flow channel.

Figure 27 shows a cross-sectional view of yet another embodiment of a damper structure in accordance with the present invention, wherein damper structure 2700 comprises portion 2702a of flow channel 2702 having a larger cross-section, an upper region of portion 2702 being filled with air or some other type of fluid 2704. As material 2706 flowing through channel 2702 experiences pressure oscillations 2708 and eventually encounters damper 2700, energy is expended as material 2706 pushes against fluid 2704. This expenditure of energy serves to reduce the amplitude of energy of oscillation of material in the flow channel.

Figure 28 shows a plan view of yet another embodiment of a damper structure in accordance with the present invention. Damper structure 2800 comprises the combination of cavity 2802 overlying and separated from underlying flow channel 2804 by membrane 2806, and constriction 2808 in the width of flow channel 2804 positioned downstream of cavity/membrane combination 2802/2806. In a manner analogous to operation of a resistance-capacitance (RC) element of an electronic circuit, cavity/membrane combination 2802/2806 serves as a flow capacitor while constriction 2808 serves as a flow resistor, resulting in a reduction in amplitude of pressure oscillations 2810 downstream of damper structure 2800.

While the above embodiments have described dampers that are specifically implemented as separate structures in the architecture of a microfluidic device, embodiments of the present invention are not limited to such structures. For example, the elastomer material in which flow channels are formed may itself serve to absorb pressure oscillations within the flow, independent of the presence of separate membrane/cavity structures or fluid filled portions of enlarged flow channels. The damping effect of the elastomer material upon pressure oscillations would depend upon the elasticity of the particular elastomer, and hence its ability to change shape during absorption of energy from the oscillating flow.

Damper structures in accordance with embodiments of the present invention function reduce the amplitude of oscillations by absorbing energy through displacement of a moveable portion of the damper structure, for example a flexible membrane or a fluid pocket. The damper structures will generally operate most

efficiently to reduce the amplitude of oscillations within a given frequency range. This frequency range is related to the speed of displacement and recovery of the moveable portion relative to the oscillation frequency. The speed of displacement and recovery of the moveable element is in turn dictated by such factors as material composition, and the design and dimensions of a specific damper structure.

Damper structures in accordance with embodiments of the present invention may also be utilized to create fluid circulation substructures. This is illustrated and described in conjunction with Figures 29A-29B, which show cross-sectional views of embodiments of circulation structures utilizing damper structures in accordance with the present invention.

Fluid circulation system 2900 comprises flow channel 2902 formed in elastomer material 2904 and featuring valves 2904 and 2906 positioned at either end. End valves 2904 and 2906 are actuated, such that elastomer valve membranes 2904a and 2906a project into and block flow channel 2902, forming sealed flow channel segment 2902a.

Pump 2908 and damper 2910 are positioned adjacent to sealed flow segment 2902a. Pump 2908 comprises recess 2908a separated from underlying flow channel 2902 by pump membrane 2908b. Damper 2910 comprises cavity 2910a separated from underlying flow channel 2902 by damper membrane 2910b.

As shown in Figs. 29A-B, pump 2908 and damper 2910 cooperate to permit a continuous circulation of fluid within sealed segment 2902a. Specifically, pump 2908 is first actuated such that pump membrane 2908b deflects into flow channel 2902, increasing the pressure within sealed segment 2902a. In response to this increased pressure, damper membrane 2910b is displaced into cavity 2910a and fluid within segment 2902a circulates to occupy the additional space.

Subsequently, pump 2908 is deactuated such that pump membrane 2908b relaxes to its original position, out of flow channel 2902. Because of the reduced pressure experienced by segment 2902a as a result of the deactuation of pump 2908, damper membrane 2910b also relaxes back into its original position, displacing material back into flow channel 2902. As a result of this action, the material within sealed segment 2902a experiences a back flow, and circulation is accomplished.

The circulation of material as just described may prove useful in a number of applications. For example, where a mixture comprising several components is being manipulated by a microfluidic apparatus, the circulation may serve to ensure homogeneity

of the mixture. Similarly, where a suspension is being manipulated, the circulating action may serve to maintain particles in suspension.

Moreover, certain components of a fluid being manipulated by a microfluidic device, such as cellular material, may stick to flow channel sidewalls. Maintenance of a continuous circulation within the flow channels may help prevent loss of material to the channel walls.

III. Sorting Applications

In one particular embodiment of the present invention, the microfluidic device comprises a T-channel for sorting materials (e.g., cells or large molecules such as peptides, DNA's and other polymers) with fluid flow channel dimensions of about 50 μm x 35 μm (width x depth). The width of pressure channels (i.e., pneumatic pump) and the damper is 100 μm and 80 μm, respectively. The gap between the flow channel and the damper (or the pressure channel) is about 5 to 6 μm. In order to produce such a thin first layer, the MLSL process requires providing a layer of an elastomer (e.g., by spreading) which is typically thinner than most other previously disclosed microfluidic devices. For example, when using GE RTV 615 PDMS silicon rubber, previous microfluidic devices typically used 30:1 ratio of 615A:615B at 2000 rpm spin-coating to fabricate the first (i.e., bottom) layer of the elastomer and 3:1 ratio of A:B for the second elastomer layer. However, it has been found by the present inventors that the silicon rubber does not cure when the ratio of 30:1 is used in fabricating the above described dimensions of fluid flow channels in the first elastomer layer.

Moreover, in order to produce a thin first elastomer layer, a higher spin-coating rate was required. For example, without using any diluent, GE RTV 615 PDMS silicon rubber A and B components in the ratio of about 20:1 was required at 8000 rpm to produce the first elastomer layer having about 3.5 μm flow channel depth and about 5-6 μm thickness between the flow channel and the damper (or the pressure channels). When SF-96 diluent was used, spin-coating at about 3000 rpm can be used to achieve a similar dimension first elastomer layer.

During fabrication of a mold, the photoresist is typically etched using a mask, developed and heated. Heating of the developed photoresist reshapes trapezoid-shaped "ridges", which ultimately form the channels, to a smooth rounded ridges and reduces the height of ridges from about 20 μm to about 5 μm. This method, however,

does not provide channels having depth of about 3.5 μm. The present inventors have found that this limitation can be overcome by treating the developed photoresist with oxygen plasma (e.g., using SPI Plasma Prep II from SPI Supplies a Division of Structure Probe, Inc., West Chester, PA) and heating the photoresist at a lower heat setting. Unlike previous methods, where a higher heat setting appear to chemically modify the photoresist, the lower heat setting used in the present invention does not chemically alter the photoresist.

Microfluidic devices of the present invention can be used in a variety of applications such as sorting cells as disclosed in commonly assigned U.S. Patent Application Serial No. 09/325,667 and the corresponding published PCT Application No. US99/13050, and sorting DNA's as disclosed in commonly assigned U.S. Patent Application Serial No. 09/499,943, all of which are incorporated herein by reference in their entirety.

The actual dimensions of a particular microfluidic device depend on its application. For example, for sorting bacteria which typically have cell size of about 1 μm, the width of fluid flow channel is generally in the range of from about 5 μm to about 50 μm and the depth of at least about 5 μm. For sorting mammalian cells which have typically have cell size of about 30 μm, the width of fluid flow channel is generally in the range of from about 40 μm to about 60 μm and the depth of at least about 40 μm. For DNA sorting, the dimensions of fluid flow channels can be significantly less.

TABLE A below provides a nonexclusive, nonlimiting list of candidate sortable entities, their approximate size range, and the approximate range of flow channel widths of a microfluidic apparatus at the point of detection of the entity.

TABLE A

SORTABLE ENTITY	APPROXIMATE SIZE RANGE OF SORTABLE ENTITY (μm)	APPROXIMATE RANGE OF FLOW CHANNEL WIDTH AT DETECTION POINT (μm)
bacterial cell	1-10	5-50
mammalian cell	5-100	10-500
egg cell	10-1000	10-1000
sperm cell	1-10	10-100
DNA strands	0.003-1	0.1-10
proteins	0.01-1	1-10

micelles	0.1-100	1-500
viruses	0.05-1	1-10
larvae	600-6500	VARIABLE
beads	0.01-100	VARIABLE

The information presented in TABLE A is exemplary in nature, and is intended only as a nonexclusive listing of candidates for sorting utilizing embodiments in accordance with the present invention. The sorting of other entities, and variation in approximate channel widths utilized to sort the entities listed above, are possible and would vary according to the particular application.

One particular embodiment of the present invention is shown in Figures 30A and 30B, where Figure 30A is a schematic drawing of the microfluidic device shown in Figure 30B. In this embodiment, the microfluidic device comprises an injection pool 3022, where a fluid containing a material can be introduced. The fluid is then pumped through the fluid flow channel 3034 via a pneumatic pump 3010 which comprises three pressure channels. By alternately pressurizing the three pressure channels, one can pump the fluid through the fluid flow channel 3034 in a similar fashion to a peristaltic pump.

The fluid exiting the pump oscillates due to actions of the pump. The fluid oscillation amplitude is attenuated by dampers 3014 which is located above the flow channel 3034, behind the pump 3010 and before a detector (not shown). Initially, the collection valve 3018A is closed and the waste valve 3018B is open to allow the fluid to flow from the injection pool 3022 through the T-junction 3038 and into the waste pool 3030. When a desired material is detected by the detector (not shown), the waste valve 3018B is closed and the collection valve 3018A is opened to allow the material to be collected in the collection pool 3026. The valves 3018A and 3018B are interconnected to the detector through a computer or other automated system to allow opening and closing of appropriate valves depending on whether a desired material is detected or not.

Dimensions of the embodiment shown above in Figs. 30A and 30B are as follows. The width of the peristaltic pumps is 100 μm. The width of the dampers is 80 μm. The width of the switch valves is 30 and 50 μm. The dimensions of the T channel is 50 x 3.5 μm. The dimensions at the T-junction are 5 x 3.5 μm. The thickness of the interlayer is 6 μm.

One such application for the microfluidic device described above is in a reverse sorting of a material (e.g., beads, DNA's, peptides or other polymers, or cells) as shown in Figures 31A and 31B. In the reverse sorting process, the material is allowed to flow towards the waste pool 3030 as shown in Figure 31A. When a desired material is detected by a detector (not shown) the pump (not shown) is reversed until the material is again detected by the detector. At this point, the waste valve 3018B is closed and the collection valve 3018A is opened, as shown in Figure 31B, and the flow of material is again reversed to allow the material to flow into the collection pool 3026. After which the collection valve 3018A is closed and the waste valve 3018B is opened. This entire process is repeated until a desired amount of materials in the injection (or input) pool 3022 is "sorted". The reverse movement of materials in the flow channel as just described can be assisted by changing pressures applied directly to flow channel inlets and outlets.

In one embodiment of the present invention, *E. Coli* expressing GFP is sorted using the reversible sorting process described above. As shown in Figures 32A and 32B, the cell velocity depends on the frequency of the pump. Thus, the cell velocity reaches a maximum of about 16 mm/sec at about 100 Hz of pump rate. Moreover, as expected, the mean reverse time in Figure 31B, which represents the time interval between detection of *E. Coli* expressing GFP, reversing the pump, and detection of the same *E. Coli*, decreases as the pump frequency is increased.

In another embodiment of the present invention provides sorting materials according to ratio of wavelengths (e.g., from laser induced fluorescence). For example, by measuring two different fluorescence wavelengths (e.g., $\lambda 1$ and $\lambda 2$) and calculating the ratio of $\lambda 1$ and $\lambda 2$, one can determine a variety of information regarding the material, such as the life cycle stage of cells, the stage of evolution of cells, the strength of enzyme-substrate binding, the strength of drug interactions with cells, receptors or enzymes, and other useful biological and non-biological interactions.

Another embodiment of the present invention provides multiple interrogation (i.e., observation or detection) of the same material at different time intervals. For example, by closing of the valves 3018A or 3018B in Figure 33C and alternately pumping the fluid to and from the input well 3022 at a particular intervals, the material can be made to flow to and from the input well 3022 through the detector (not shown). By oscillating this material through the detection window 3040, one can observe

the material at different time intervals. For example, a sample can be interrogated at 10 Hz pump frequency as shown in Figure 33A or at 75 Hz pump frequency as shown in Figure 33B. As expected, at a higher pump frequency, the material can be observed at shorter intervals. Such observation of materials at different time has variety of applications including monitoring cell developments, enzyme-substrate interactions, affect of drugs on a given cell or enzyme; measuring half-life of a given material including drugs, compounds, polymers and the like; as well as other biological applications.

The performance of a sorter structure in accordance with embodiments of the present invention may be dependent upon the elastomer material utilized to fabricate the device. Fig. 34 plots flow velocity versus pump frequency for cell sorters fabricated from different elastomeric materials, namely General Electric RTV 615 and Dow Corning Sylgard 184.

Fig. 34 shows that the flow velocity of cells through the cell sorters reached a maximum at a pumping frequency of about 50 Hz. The decline in flow velocity above this frequency may be attributable to incomplete opening and closing of the valves with each pumping cycle.

Moreover, different values for maximum pumping rates of the two sorting structures are different. The RTV 615 cell sorter exhibits a maximum pumping rate of about 10,000 μm/sec, while the Sylgard 184 cell sorter exhibits a maximum pumping rate of about 14,000 μm/sec. Maximum flow rates of other elastomeric microfluidic devices in accordance with the present invention have ranged from about 6000 μm/sec to about 17,000 μm/sec, but should be understood as merely exemplary and not limiting to the scope of the present invention.

Fig. 34 indicates that the pumping rate of a cell sorter device may be controlled by the identity, and hence flexibility, of the particular elastomer used. In the instant case, based upon the relative flexibility of RTV 615 and Sylgard 184, the greater the elasticity of the elastomer results in a faster rate of pumping.

Other changes, for example the addition of diluents to the elastomer or the mixing of different ratios of A and B components of fluidic layer, may allow even further fine tuning of the pumping rate. Changing the dimensions of the fluidic channel may also allow tuning of the pumping rate, as different volumes of fluid in the channels will be moved with each actuation of the membrane.

While the sorting device described above utilizes a T-shaped junction between flow channels, this is not required by the present invention. Other types of junctions, including but not limited to Y-shaped, or even junctions formed by the intersection of four or more flow channels, could be utilized for sorting and the device would remain within the scope of the present invention.

Moreover, while only a single sorting structure is illustrated above, the invention is not limited to this particular configuration. A sorter in accordance with embodiments of the present invention is readily integratable with other structures on the same microfabricated device. For example, embodiments in accordance with the present invention may include a series of consecutively-arranged sorting structures useful for segregating different components of a mixture through successive sorting operations.

In addition, a microfluidic device in accordance with embodiments of the present invention could also include structures for pre-sorting and post-sorting activities that are in direct fluid communication with the sorter. Examples of pre- or post-sorting activities that can be integrated directly into a microfluidic device in accordance with the present invention include, but are not limited to, crystallization, cell lysis, labeling, staining, filtering, separation, dialysis, chromatography, mixing, reaction, polymerase chain reaction, and incubation. Chambers and other structures for performing these activities can be integrated directly onto the microfabricated elastomeric structure.

IV. Cytometry

Recently, there has been a growing interest in microfluidic flow cytometry. Several chip based systems have been demonstrated for cytometry and sorting of cells and molecules. *See, for example*, Unger et al., *Science*, **2000**, *288*, 113-116; Fu et al., *Nature Biotechnol.*, **1999**, *17*, 1109-1111; Quake et al., *Science*, **2000**, *290*, 1536-1540; Schrum et al., *Anal. Chem.*, **1999**, *71*, 4173-4177; Knight et al., *Phys Rev. Lett.*, **1998**, *80*, 3863-3866; Chou et al., *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 11-13; Chou et al., *Electrophoresis*, **2000**, *21*, 81-90; and Kameoka, et al., *Sensors and Actuators B*, **2001**, *77*, 632-637. There are generally two ways to pump fluid in these devices: pressure driven flow or by electroosmotic forces. Pressure driven flow results in Poiseulle flow, which has a parabolic velocity distribution in the channel. This complicates measurement of analytes since each analyte, e.g., cell or molecule, passes through the interrogation region with a different velocity. One can mitigate the effects of the Poiseulle flow by using a sheath fluid for hydrodynamic focusing, but this introduces other issues by

diluting the sample and complicating downstream analysis. Although electroosmotic flow is typically more uniform and plug-like than pressure driven flow, it too results in variability in flow velocity. *See, for example*, Schrum et al., *Anal. Chem.*, **1999**, *71*, 4173-4177. In addition, most, if not all, electroosmostic flow requires careful balancing of the ions in the solution and attention to prevent ion depletion. Furthermore, in some cases, it has also been shown that eukaryotic cells are difficult to manipulate electroosmotically. See, for example, Li et al., *Anal. Chem.*, **1997**, *69*, 1564-1568.

One possible method for measuring a velocity independent characteristic parameter, e.g., fluorescence, of an analyte is to use a uniform detection zone, e.g., excitation region, large enough to illuminate the entire particle or molecule of interest. In this case, the height of the detected fluorescent peak will be substantially proportional to the fluorescence intensity of the particle. *See, for example*, Chou et al., *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 11-13. While this method is substantially not affected by the distribution of velocities, using only one point from the entire peak, namely its maximum, exploits only a small part of the information that is embedded in the peak. Moreover, the accuracy of this method is susceptible to noises. On the other hand, measuring the area underneath the entire peak, which is proportional to total fluorescence intensity integrated over the excitation duration, is velocity dependent measurement. This is due to the fact that faster particles have narrower peaks than slower particles. This will result in integrals being inversely proportional to the velocity of the particle. One solution is to normalize the area of each peak by the velocity of the corresponding particle to obtain velocity independent measurement of the fluorescence intensity.

Several methods for measuring the velocity in microfluidic devices have been reported. In particle image velocimetry, video imaging is used to measure the velocities of particles in a channel by observing the displacement of the particles within a known time interval. See, for example, Singh et al., *Anal. Chem.*, **2001**, *73*, 1057-1061; Barker et al., *Anal. Chem.*, **2000**, *72*, 5925-5929; and Santiago et al., *Experiments in Fluids*, **1998**, *25*, 316-319. This method is advantageous in obtaining the velocity spatial distribution, however it is not suitable for accurately measuring other analyte characteristic parameters, such as the fluorescent intensity.

Shah convolution Fourier transform is another method to measure velocity in microfluidic devices. See, for example, Kwok et al., *Anal. Chem.*, **2001**, *73*, 1748-1753 and Crabtree et al., *Anal. Chem.*, **1999**, *71*, 2130-2138. In this method, a mask with a periodic array of slits spatially modulates the excitation beam. When an analyte is

moving in the beam the spatial modulation is converted into a temporal modulation. The distribution of velocities is found by Fourier transforming the temporal signal and identifying the peaks. However, such a practical use of this method has not been demonstrated. Moreover, the practical implementation of this method most likely will require fabrication of the mask on the chip which adds to the complexity of the device.

Therefore, there is a need for apparatuses and methods for determining a velocity independent analyte characteristic parameter.

Embodiments of the present invention relate to an apparatus and a method for determining a characteristic parameter of an analyte in a fluid medium. In one particular aspect, the present invention relates to a velocity independent flow cytometry.

One aspect of the present invention provides an apparatus for determining a characteristic parameter of an analyte in a fluid medium independent of the flow velocity of the analyte, said apparatus comprising:

(a) a device comprising a fluid flow channel;

(b) a means for transporting a fluid medium from a first position to a second position of the fluid flow channel;

(c) a plurality of detection zones located at different positions along said fluid flow channel and located in between the first and the second position of the fluid flow channel;

(d) a detector for detecting the analyte flowing through the detection zone; and

(e) a means for measuring a characteristic parameter of the analyte independent of the flow velocity of the analyte through the plurality of detection zones.

Preferably, the device which comprises a fluid flow channel is a microfluidic device. While the fluid medium can be transported through the fluid flow channel by any conventional means, preferably means for transporting a fluid medium comprises a peristaltic pump or electroosmosis. More preferably, the microfluidic device comprises a peristaltic pump. Such microfluidic devices are particularly advantageous because the median fluid medium flow velocity can be controlled.

In one particular embodiment, the detector comprises a laser, a laser beam guiding device and a fluorescence measuring device. Preferably, the laser beam guiding device is an acousto-optic modulator. In this manner, a velocity independent integrated fluorescence peak area of the analyte can be determined.

Another aspect of the present invention provides a method for determining a velocity independent characteristic parameter of an analyte, wherein the characteristic parameter of the analyte is capable of being influenced by or dependent on the velocity of the analyte, said method comprising:

(a) providing a means for transporting a fluid medium comprising the analyte from a first position to a second position of a fluid flow channel of a fluidic device;

(b) measuring the characteristic parameter of the analyte within the fluid flow channel at a plurality of locations along the fluid flow channel in between the first and the second position; and

(c) determining the velocity independent characteristic parameter of the analyte using the measured characteristic parameters of step (b) and normalizing the measurement by substantially eliminating the velocity component of the measurement.

The velocity independent characteristic parameter of the analyte can be determined by:

(i) comparing signals obtained from the plurality of locations along the fluid flow channel in step (b);

(ii) determining a time difference by calculating the time it takes for a particular analyte to pass from a first detection position to a second detection position; and

(iii) determining the velocity independent characteristic parameter using the time difference.

Preferably, the signals from the first and the second detection zones are averaged and normalized using the time difference. By averaging the signals from the two detection zones, the amount of noise and false signals are significantly reduced by the methods of the present invention.

Apparatuses and methods of the present invention can be used in a variety of assay and analytical applications. In one particular embodiment, methods of the present invention provide cell sorting. Yet in another embodiment, methods of the present invention provide determining number of nucleotides present in an oligonucleotide. Such methods for determining the number of nucleotides in an oligonucleotide comprise:

(A) attaching a fluorescent molecule to the oligonucleotide to produce a modified oligonucleotide prior to measuring velocity independent characteristic

parameter of the modified oligonucleotide, wherein said characteristic parameter is integrated fluorescent peak area of said modified oligonucleotide; and

(B) determining the number of nucleotides in the oligonucleotide by comparing the velocity independent integrated fluorescence peak area of the modified oligonucleotide with a velocity independent fluorescence peak area of a standard oligonucleotide, wherein the velocity independent fluorescence peak area of the standard oligonucleotide has been calibrated to the number of nucleotides present the standard oligonucleotide.

In the present application, the term "Analyte" refers to any material in a fluid medium which can be analyzed using a detector. Exemplary analytes include cells; oligonucleotides, such as DNA, RNA and PNAs; other organic compounds, such as pharmaceutically active compounds including antibiotics, antiviral compounds, anticancer compounds, etc.; beads; resins; polymers; and the like.

The terms "character of an analyte," "character parameter of an analyte" and "an analyte's characteristic" are used interchangeably herein and refer to a physical or chemical characteristic parameter of the analyte. Exemplary analyte's characteristics include molecular size (e.g., weight or length), fluorescence, infrared or UV/Vis absorption, nuclear magnetic resonance (i.e., NMR) spectrum, cell type, and other characteristics which can be measured or detected by a suitable detector known to one skilled in the art.

The terms "fluid" and "fluid medium" are used interchangeably herein and refer to a gas, or preferably liquid.

The terms "velocity" and "flow velocity" when referring to an analyte are used interchangeably herein and refer to the flow velocity of the analyte within a fluid medium.

The present invention provides an apparatus for determining a plurality of characteristic parameters of an analyte in a fluid medium. In particular, the apparatus of the present invention allows determination of an analyte characteristic parameter and the analyte flow velocity within a fluid flow channel of a device. Preferably, the analyte characteristic parameter is capable of being influenced and/or affected by the analyte flow velocity. In this manner, the apparatus of the present invention provides or can be used to determine a velocity independent analyte characteristic parameter.

The analyte characteristic parameter can be any physical or chemical parameter that can be measured or determined using conventional detecting means.

Suitable analyte characteristic parameters include UV/VIS absorption, fluorescence, nuclear magnetic resonance, infrared spectrum, and other physical or chemical parameters which can be measured as an integrated area under a peak or a curve. For the sake of brevity and clarity, the present invention will now be described in reference to measuring fluorescence of the analyte. Of course in order to measure fluorescence, the analyte must be capable of fluorescing when exposed to an appropriate electromagnetic radiation, e.g., ultraviolet and/or visible light. Thus, in one aspect the analyte comprises a fluorescent moiety.

The total fluorescence intensity integrated over the excitation duration is velocity dependent measurement. As expected, when fluorescence of analytes is measured in a narrow detection zone (i.e., area), analytes with faster flow velocity spend a relatively small amount of time within the detection zone resulting in a relatively smaller fluorescence intensity peak width compared to slower analytes. For example, as illustrated in Figure 35A, at a similar velocity the peak intensity and the peak area is proportional to the length of the DNA being detected by flow cytometry. However, as shown in Figure 35B, if two similar length of DNAs have different velocity, the faster moving DNA will have smaller peak area (in Figure 35B, the dotted line shows that the peak heights of both particles are the same) and the slower moving DNA will have larger peak area. This velocity difference can lead to misleading or erroneous interpretation flow cytometry data.

The apparatuses and methods of the present invention significantly reduce or eliminate the affect of molecule velocity through a detection zone on the peak area by eliminating or normalizing the velocity factor of the analyte characteristic parameter. In particular, apparatuses of the present invention comprise at least two different detection zones along the analyte's flow path (i.e., fluid flow channel) to determine the velocity of each analyte that flows through the detection zones. By placing two different detection zones at a predetermined distance (i.e., "d") from each other, one can measure the velocity of the analyte flowing through the detection zones by measuring the time difference (i.e., "t") at which the analyte passes through the first detection zone and the second detection zone. Since the velocity (i.e., "v") is distance divided by time, the flow velocity of analyte is calculated by the formula: $v=d/t$, where v, d and t are those defined above. The peak area is then multiplied by the velocity (or simply divided by time since d is constant) to normalize the peaks, i.e., to eliminate the velocity factor. In this manner, a more accurate determination of the analyte characteristic parameter can be made.

Preferably, each analyte passes through the detection zone individually, i.e., separately. Therefore, the variables such as flow channel width, concentration of the analyte, and other variables which can affect the number of analyte passing through the detection zone is adjusted such that a single analyte flows through the detection zone at any given time. For measuring characteristic parameters of cells, the width of fluid flow channel is preferably in the order of from about 1 μm to about 1000 μm, more preferably from about 10 μm to about 100 μm, and still more preferably from about 5 μm to about 50 μm.

For example, for measuring characteristic parameters of a relatively large molecules having molecular weight of about 3 KDaltons, such as oligonucleotides including DNA's, RNAs, PNAs and hybrids thereof; peptides; polymers; and other organic compounds, the width of the fluid flow channel is preferably in the range of from about 1 μm to about 50 μm, and more preferably from about 3 μm to about 10 μm. While the above fluid flow channel width are provided as being particularly suitable for certain analytes, it should be appreciated that other fluid flow channel widths are also within the scope of the present invention.

Devices comprising such fluid flow channel dimensions are well known to one skilled in the art. For example, any microfluidic devices currently known to one of ordinary skill in the art can be used in the present invention. However, preferred microfluidic devices are constructed of single or multilayer soft lithography (MLSL) as described by Unger et al. in *Science*, **2000**, *288*, 113-116, and further detailed in commonly assigned U.S. Patent Application Serial No. 09/605,520, filed June 27, 2000, which are incorporated herein by reference in their entirety. Other preferred microfluidic devices are disclosed in a commonly assigned U.S. Patent Application entitled "Microfluidic Devices and Methods of Use," which is filed even date with the present application and is further identified by attorney docket No. 020174-002510US, and is incorporated herein by reference in its entirety. Moreover, specific examples of microfluidic flow cytometry for sorting cells and DNA's are disclosed in commonly assigned U.S. Patent Application Serial No. 09/325,667 and the corresponding published PCT Patent Application No. US99/13050, and U.S. Patent Application Serial No. 09/499,943, respectively, all of which are incorporated herein by reference in their entirety.

While one can use multiple detectors and electromagnetic radiation sources (e.g., laser for laser induced fluorescence), it has been found by the present inventors that one or more, preferably two, acousto-optic modulators in conjunction with an aperture is particularly suitable for providing two different detection zones from a single laser source. An acousto-optic modulator is readily available from a variety of sources including at http://www.brimrose.com/acousto_modulators.html, which also includes a general discussion on the theory behind acousto-optic modulators (Brimrose Corp., Baltimore, Maryland). Other devices which is capable of guiding the laser beam into two or more different positions can also be used instead of an acousto-optic modulator. Such devices are well known to one of ordinary skill in the art and include rotating mirrors, gratings and other electromagnetic wave diffracting devices. The aperture allows emission of only one particular diffracted beam to illuminate the detection zones and blocks other diffracted laser beam.

When using an acousto-optic modulator, preferably the first order beam is used. Thus, when two acousto-optic modulators are used, one to control direction of the laser beam in the x-axis and the other to control direction of the laser beam in the y-axis, the resulting laser beam is about 20% intensity of the original laser beam. This is because a typical first order laser beam intensity exiting an acousto-optic modulator is about 50% of the laser beam entering it. Therefore, by using two acousto-optic modulator, the resulting laser beam is about 20% to about 30% intensity of the original laser beam.

As stated above, an aperture is typically used in conjunction with acousto-optic modulators. The aperture is used to select the first order laser beam from each of the acousto-optic modulators. For example, as shown in Figure 36C, there are variety orders of diffracted laser beam resulting form two acousto-optic modulators. The aperture allows laser beam from only the first order of both acousto-optic modulator (i.e., 50) to be focused onto the detection zones.

A typical set-up for using a laser to detect fluorescence of the analyte in two detection zones is schematically illustrated in Figure 36A. The laser beam enters two orthogonally located acousto-optic modulators 1100. The aperture 1104 then allows only the first order laser beam from both of the acousto-optic modulators to pass through. The resulting laser beam then passes through two lenses 1108A and 1108B. However, the use of two lenses is optional and can be omitted. The laser beam is then deflects off the dichroic beam-splitter 1112 and is focused on to the detection zones through an objective 1116. The fluorescence wavelength then passes through a filter 1120 to a channel photo-

multiplier 1124, which is operatively interconnected to a data acquisition device 1128. The optional oscilloscope 1132 can be used to observe the signal prior to data acquisition and/or to observe the signal in real-time.

The result of one particular embodiment of the present invention is shown in Figure 36B. The left portion of Figure 36B shows a T-shaped flow channel which is illuminated with fluorescence at two different detection zone of the flow channel. The right portion of Figure 36B shows signal peaks corresponding to the square wave which controls the x-axis position, i.e., the detection zone, of the laser beam and the fluorescence peaks obtained from the two detection zones. In this embodiment, the distance 110 between the two detection zones 114L and 114R is about 10 μm and the width of the fluid flow channel is about 5 μm. The laser beam enters an acousto-optic modulator and the first order beam is emitted through an aperture. In order to scan the entire cross section of the fluid flow channel and to allow scan of two different detection zones 114L and 114R, two acousto optic modulators are used. One to control the x-axis and the other to control the y-axis of the laser beam. In Figure 36B, the beam has y-axis frequency of 150 kHz, i.e., the beam travels from above the "top" of the flow channel 120 to below the "bottom" of the flow channel 124 at a rate of 150,000 times per second. In actuality the beam scans twice the distance relative to the width of the fluid flow channel; however, because there is no fluorescence outside the fluid flow channel, no beam is visible outside the fluid flow channel in Figure 36B. In addition, it has a sampling rate of 40 kHz, i.e., each y-position is sampled about 4 times (150/40). Furthermore, the laser beam switches from the detection zone 114L to 114R and vice a versa at a rate of 5 kHz. Frequency of x-axis switching can be seen in the top graph of the right portion of Figure 36B. In this graph, when the peak is at the top, it represents detection (or scanning) in the 114R region, and when the peak is at the bottom (i.e., 0) it represents detection (or scanning) in the 114L region. As can be seen, the laser beam moves from one position to another (in the x-axis) to allow scanning of two different positions. This allows the same analyte to be detected at two different times at two different regions as shown in the lower graph of the right portion of Figure 36B. By determining the time difference between such detection and knowing the distance 110 (Figure 36A), one can calculate the velocity of the material traveling through the fluid flow channel. As stated above, it is preferred that statistically each analyte enters the detection zone separately.

As stated above, the left portion of Figure 36B illustrates scanning two detection zones of a T-shaped fluid flow channel by the scanning beam. A fluid medium comprising fluorescein was introduced to the fluid flow channel and was excited by the scanning beam in two scan lines as shown. The resulting fluorescence was imaged with a CCD camera as shown in Figure 36B. The two scan lines are clearly visible. It is important to note that the two scan lines can be seen together only due to the limited time resolution of the CCD compared to the scanning frequency. In actuality, the two bright lines of Figure 36B are actually fluorescing at different times. The lines scanned by the illuminating beams are about two times longer than the bright lines shown in Figure 36B to ensure uniform excitation of the region of interest. However, the full line can not be seen because there is no fluorescein outside of the fluid flow channels, and therefore no fluorescence occurs there. The width of the fluid flow channel in Figure 36B is 5 μm and its depth is 3.9 μm.

As shown in Figure 36A, in one embodiment the laser beam is transmitted through two mutually orthogonal acousto-optic (AO) modulators, together represented as item 1100, to produce two line scans. Each AO was driven by voltage controlled oscillator (VCO) connected to a function generator. As implied above, one of the AO is used to move the beam in the x direction, i.e., parallel to the direction of the fluid flow. Typically, this VCO was driven with a square wave (e.g., 5KHz, $7V_{p-p}$) thereby allowing one to scan two different detection zones along the fluid flow channel. Because the laser beam scans only a small fraction of the width of the fluid flow channel, a second AO is required to scan the entire width of the fluid flow channel. This second AO is preferably oriented to enable the laser beam to move in the Y direction, thus allowing the beam to cross the entire width of the fluid flow channel. Typically, the signal for controlling this second AO is a sinusoidal wave (e.g., 120KHz, $9V_{p-p}$) at a frequency higher than the chopping signal to ensure several crossing of the channel for each position on the x direction. At the exit of the second AO the original laser beam is divided into a grid of beams as illustrated in Figure 36C. Most of these beams are blocked using an aperture 1104 leaving only the beam that is the combination of the first orders of both AOs. This beam is then used to scan along two parallel lines (left and right line scans) in different positions along the fluid flow. Such scanning can be conveniently accomplished by a VCO. As stated above, the data acquisition can be conveniently accomplished by a channel photo-multiplier 1124 operatively connected to a data acquisition device, such as

a computer 1128 or other signal recording device, including a plotter. Typically, the two signals, the square wave used to control AOs and the fluorescence signal, are recorded separately, i.e., as Ch1 and Ch2 in Figure 36B. By correlating signals of Ch2 to the square wave of Ch1 allows one to determine which peaks in Ch2 are from the left (114L) detection zone and which peaks are from the right (114R) detection zone.

By normalizing the total peak area (from both 114L and 114R regions) in the graph of Figure 36B (i.e., Ch2), one can plot a new normalized graph as shown in Figure 37. As the legends in Figure 3 shows, the graph represents peaks from the left (114L) and right (114R) detection zones and a normalized graph (i.e., labeled as "both"). Alternatively, one can average the two normalized peak areas (from 114L and 114R regions). It has been found that in some cases averaging the normalized peak areas can reduce the coefficient of variance relative to using only a single detection zone.

It has been found by the present inventors that because apparatuses of the present invention provide a plurality of detection zones, the resulting peak area (i.e., characteristic parameter) that has been normalized have a significantly lower coefficient of variance compared to conventional apparatuses having only one detection zone. Typically, apparatuses and methods of the present invention improves the coefficient of variance by a factor of at least about 2 relative to a similarly equipped apparatus having only one detection zone, and preferably by a factor of at least about 3.

Gel-electrophoresis (i.e., electrophoresis) and other similar methods have limited resolution capacity for medium to large DNA molecules, and therefore are inapplicable in many cases. In contrast, apparatuses and methods of the present invention are not limited by the size of material (e.g., DNA). Moreover, if the distance between two detection zones are large or the velocity of the material is slow, one can use these variations to study a variety of analyte characteristic parameters. For example, one can detect changes in cells as it passes through from one detector to another. One can also analyze chromosome distribution in cells (e.g., karyotyping). Methods of the present invention are also useful in epidemiology and other diagnostic and assay procedures.

In one particular aspect, apparatuses of the present invention can be used to determine the size (e.g., number of base pairs) of oligonucleotides such as DNAs, RNAs, PNAs and hybrids thereof. For example, a calibration chart can be prepared by measuring fluorescence of oligonucleotides of a variety of lengths. To be useful, each oligonucleotides are coupled to a compound that is capable of fluorescing. The fluorescence compound is then coupled to the oligonucleotide at a regular interval, e.g.,

every four or five base pairs. The oligonucleotide of unknown length is then coupled to the same fluorescence compound at the same nucleotide interval. By measuring the fluorescence peak area and comparing the result with the calibration chart, e.g., by a computer, one can easily determine the length of the unknown oligonucleotide. Thus, apparatuses and methods of the present invention can be used as an alternative to electrophoresis to determine the size of oligonucleotides. However, unlike electrophoresis, apparatuses and methods of the present invention are generally not limited by the size of the oligonucleotide.

Apparatuses and methods of the present invention allow determination of analyte flow velocity and other characteristic parameter(s) of the analyte. For example, such apparatuses and method can be used to perform flow cytometry and determine the analyte flow velocity; thereby, enabling correlation of the velocity with each specific analyte. In addition, usefulness of apparatuses of the present invention is not limited by the size of the analyte. In contrast, measuring the fluorescence from the peak heights using a conventional apparatus typically requires the entire particle (i.e., analyte) to be uniformly illuminated by the excitation beam, thereby limiting the size of the particle which can be analyzed.

1. Acquisition and Analysis

In one particular embodiment of the present invention, the data acquisition involves scanning two different detection zones along the path of the fluid flow channel by AOs which are operatively interconnected to VCOs. Each detection zone is scanned at least the entire width of the fluid flow channel. Typically, each acquisition involves four or more scans per detection zone. That is, the laser beam scans "up and down" the width of the detection zone (i.e., y-axis of the fluid flow channel) four or more times before the x-axis position (i.e., detection zone) of the beam is switched. Data acquisition typically involves recording the fluorescence signal in each of the two detection zones and separately recording the square wave that is used to direct the laser beam from to different detection zones. If the peak and the valley of the square wave represent relative values 1 and 0, respectively, typically 0.05 and 0.95 fractions of square wave signals are used as the locations for the first and the second detection zones. This eliminates any noise factor that may be present in the signal, e.g., due to signal spikes.

As stated above, data acquisition involves recording the square wave frequency and the fluorescence intensity of the analyte. By correlating the square wave to

the fluorescence peaks, one can determine whether a particular fluorescence peak is from the first or the second detection zone. For example, if the laser beam is directed at the first detection zone near the peak of the square wave, then any fluorescence peaks occurring near the peak of the square wave is due to fluorescence at the first detection zone. Conversely, any fluorescence peaks occurring near the bottom of the square wave is due to fluorescence at the second detection zone. In this manner, the fluorescence signal can be separated into its two origin signals (one for each detection zone) even in cases where a particle is long enough to be in both detection zones at the same time. This type of data analysis algorithm allows detection of fluorescence from both detection zones using a single detector.

After each peak from the first detection zone has been correlated to a corresponding peak from the second detection zone, the peaks are then normalized by dividing the integrated peak area with the analyte flow velocity. The analyte flow velocity can be determined by correlating each of the first and the second detection zone to the square wave signal. Since the distance between the first and the second detection zone is constant, the integrated peak area can be simply divided by the time it take for the analyte to travel from the first detection zone to the second detection zone. Because each integrated peak area from the first detection zone is correlated to the corresponding integrated peak area from the second detection zone, any integrated peak area from the first detection zone that does not have a corresponding integrated peak area in the second detection zone or vice a versa is most likely due to noise and is discarded. Thus, apparatuses and methods of the present invention provide more accurate analyte characteristic parameter determination than conventional single detection methods.

The distance between the two detection zones should be sufficiently large enough to allow accurate velocity determination, i.e., time it takes for the analyte to travel from the first detection zone to the second detection zone. Generally, the accuracy of the velocity determination is somewhat dependent on separation (in time or more importantly in sampling points) between the two corresponding fluorescence peaks. Therefore, larger separation of the fluorescence peaks, i.e., longer it take for an analyte to travel from the first detection zone to the second detection zone, results in more accurate velocity determination.

In one particularly preferred embodiment, statistically only one analyte passes through both detection zones before the second particle enters the first detection zone. In this manner, the integrated peak areas from the first detection zone can be

readily correlated to the integrated peak areas from the second detection zone. This can be accomplished by adjusting one or both of the distance between two detection zones and the concentration of the analyte in the fluid medium. Of course these two parameters are also dependent on other variables such as the mean flow velocity of the fluid medium within the fluid flow channel and the square wave frequency.(i.e., frequency at which the laser beam switches to and from the first and the second detection zones).

For example, for a mean fluid medium flow velocity of about 2 μm/sec and square wave frequency of 5000 sec^{-1}, the distance between the two detection zone is about 10 μm. And the concentration of the analyte is typically from about 2 nM or less, preferably from about 0.2 nM to about 2 nM.

Theoretically, activating data acquisition prior to having any of the analyte pass through the first detection zone allows one to easily correlate each fluorescence peak to a particular particle, because the order of each peak on the first and the second detection zones will be equal to the order of particles passing through each detection zone. However, in many instances such signal acquisition is not feasible or practical. Moreover, possible noises in each of the detection zones will prevent correlating the peak from one detection zone to another detection zone difficult. Therefore, one needs to correlate the integrated peak area (e.g., fluorescence peaks) from the first detection zone to the corresponding integrated peak area of the second detection zone.

One method of achieving this correlation is to correlate the integrated peak area from the first detection zone to a corresponding integrated peak area in the second detection zone by limiting the detection to region of velocities about $\pm$ 4 times the median flow velocity of the fluid medium. For example, integrated peaks from the first detection zone is compared to integrated peaks from the second detection zones in the range of ¼ the median flow velocity to 4x the median flow velocity of the fluid medium. By comparing other integrated peak areas of similar intervals, one can verify whether the initial correlation is accurate. Such a method for detecting and correlating the integrated peak areas from the first detection zone to the integrated peak areas of the second detection zone is schematically outlined in the flow sheets in Figures 41 and 42. Briefly, the two integrated peak areas (e.g., $A_l(i)$ and $A_r(i)$) are compared to see whether they are within the minimum and the maximum time difference as specified. The minimum and maximum time differences can be adjusted depending on the median flow velocity of the fluid medium. Once a pair of matching integrated peaks from the first and the second

detection zones is found, the time difference (i.e., TimeDiff or Δt) is determined. The time difference is inversely proportional to the analyte flow velocity and is the difference in time when the particle crosses (i.e., detected by) the first and second detection zones. The velocity independent integrated peak area is then calculated by averaging the integrated peak areas of the particle from the first and the second detection zones and dividing the average integrated peak area by time difference. This calculation can be represented by the following formula:

$$A_{vi} = [(A_l + A_r)/2]/\Delta t$$

where A_{vi} is velocity independent area, A_l is integrated peak area from the first detection zone, A_r is integrated peak area from the second detection zone and Δt is time difference. In Figure 42, the time difference is determined by dividing the difference in point number of A_l and A_r with the sampling rate (i.e., the frequency of switching the laser beam to and from the first and the second detection zones).

Since the velocity independent peak area is determined by averaging the two integrated peak areas from the first and the second detection zones, the apparatuses and methods of the present invention reduces the "false signal" or the noise significantly. Moreover, any integrated peak from one detection zone that does not have a corresponding integrated peak on the second detection zone or vice versa is eliminated resulting in further reduction of noise. In conventional single detection method, such peaks are often mistaken as an actual signal, thereby resulting in misinterpretation of the data. By comparing the signals from one detection zone to the other, methods of the present invention further reduces noise or false signals.

It should be appreciated that while the present invention has been described with respect to using a single laser beam with two acousto-optic modulators to guide the laser beam into two scanning detection zones, other arrangements are also possible and are within the scope of the present invention. For example, apparatus of the present invention can be fabricated to utilize two laser beams directed at different detection zone and having a separate acousto-optic modulator for scanning the entire width of the fluid flow channel. In addition, other electromagnetic wave diffracting devices, such as rotating mirrors, gratings and other electromagnetic wave diffracting devices known to one skilled in the art, can be used instead of an acousto-optic modulator.

Moreover, the control over the line scans (i.e., detection zones), which is done by controlling the AOs, is done electronically and can be changed depending on a particular application. For example, the two line scans can be easily rotate by 90 degrees to enable the same measurement in a perpendicular channel. This general measurement method can be used to measure the velocity of particles in a variety of microfluidic devices without the need for any changes in the design of microfluidic devices.

2. Microfluidic Device

Apparatuses of the present invention also comprise a fluidic device, preferably a microfluidic device, which comprises a fluid flow channel. The fluid flow channel allows flows of the fluid medium from one location to another location within the device. Preferably, the cross-section of the fluid flow channel should be small enough such that only a very small area is scanned by a laser. In general, any microfluidic device made of a material that is transparent to the laser beam is suitable. However, a particularly preferred microfluidic devices are those disclosed by Unger et al. in *Science*, 2000, *288*, 113-116, and U.S. Patent Application Serial No. 09/605,520, which were incorporated by reference above.

In particular, microfluidic devices which comprise a peristaltic pump is particular useful in the present invention as they allow one to control the median fluid medium flow velocity. However, it should be appreciated that the fluid medium can be made to flow through the fluid channel by any of the conventional means, such as pressure gradient, electroosmotic flow, and the like.

3. Utility

Apparatuses and methods of the present invention have a wide variety of application such as flow cytometry, oligonucleotide sorting, oligonucleotide analysis, detecting changes in cells, analyzing chromosome distribution in cells (e.g., karyotyping), studying epidemiology, and other diagnostic and assay procedures. Using microfluidic devices in cell and DNA sorting are general described by Fu et al. in "A Microfabricated Fluorescence-activated Cell Sorter," *Nature Biotech.*, **1999**, *17*, 1109-1111; and Chou et al. in "A Microfabricated Device for Sizing and Sorting DNA Molecules," *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 11-13, respectively, which are incorporated herein by reference in their entirety. Methods for cell sorting using microfluidic devices are further detailed

in PCT Publication No. WO 99/61888, which is also incorporated herein by reference in its entirety.

In one particular embodiment, methods of the present invention provides determining number of nucleotides that is present in an oligonucleotide. The method generally involves modifying the oligonucleotide by attaching a fluorescent molecule to the oligonucleotide and measuring a velocity independent integrated fluorescent peak area. This velocity independent integrated fluorescent peak area is then compared with a velocity independent integrated fluorescent peak area of a standard oligonucleotide having a known number of nucleotides. Preferably, velocity independent integrated fluorescent peak areas are determined for a number of standard oligonucleotides having a different number of nucleotides. In this manner, a calibration table can be produced and used to determine the number of nucleotides present in an oligonucleotide.

The modified oligonucleotide and the standard oligonucleotides are attached with a known amount of fluorescent molecules per given number of nucleotides. In this manner, a given velocity independent integrated fluorescent peak area can be calibrated to represent a certain number of nucleotides that is present in the oligonucleotide.

Additional objects, advantages, and novel features of this invention will become apparent to those skilled in the art upon examination of the following examples thereof, which are not intended to be limiting.

4. Experimental

The fluorescence excitation was performed with a 5 mW, 488 nm laser beam from an air cooled argon ion laser (Uniphase, San Jose, CA). The laser beam was transmitted through two mutually orthogonal acousto-optic (AO) modulators to produce two line scans (see Figure.36A). Each AO was driven by voltage controlled oscillator (VCO) connected to a function generator. The first AO was oriented so as to move the beam in the x direction, parallel to the direction of the flow. Its VCO was driven with a square wave (5 KHz, 7 V_{p-p}) causing the beam to move between two points along the channel. The second AO was oriented to enable moving of the beam in the Y direction, thus allowing the beam to cross the channel. The signal that was used to control this AO was a sinusoidal wave (120 KHz, 9 V_{p-p}) at a frequency higher than the chopping signal to ensure several crossing of the channel for each position on the x direction. At the exit of the second AO the original laser beam was divided into a grid of beams. Most of these

beams were blocked leaving out only the beam that was the combination of the first orders of both AOs. This beam was thus scanned along two parallel lines (left and right line scans) in different positions along the flow. The laser beam was focused through a 100 X 1.3NA oil immersion objective (Olympus, New Hyde Park, NJ) which also was used to collect the emitted fluorescence. The full width half maximum of the beam at the channel was about 1 μm. Auxiliary lenses were used to adjust the distance between the two scan lines as well as their length to be in the order of 10 μm. The uniformity of the excitation across the channel was not dependent on the beam size but rather on the uniformity of the illumination while the beam was scanned. This was evaluated by imaging a thin layer of fluorescein in solution illuminated by the scanning beam with a CCD camera. The image was then digitized and evaluated for uniformity. A dichroic filter was used to introduce the laser light into the optical train (Chroma 500 DCLP, Chroma Technology, Brattleboro, VT). Dielectric filter was used to reduce background and scattered light from the emitted fluorescence (Chroma D535/50M). The fluorescence was imaged onto a channel photomultiplier detector (EG&G, Gaithersburg, MD). The detector output as well as the chopping square wave were digitized at a rate of 40 KHz by a National Instrument (Austin, TX) Lab PC1200 board on a personal computer running LABVIEW.

A 3.9 μm high, 5 μm wide rectangular fluid flow channel was fabricated from a silicon elastomer (General Electric RTV 615) by using a replica technique generally disclosed by Chou et al., in *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 11-13 and U.S. Patent Application Serial No. 09/605,520, filed June 27, 2000, which were previously incorporated by reference in their entirety. Master molds were made from silicon wafers by using standard micromachining techniques.

Both line scans were well inside the detecting area, thus the fluorescence signals from both line scans were recorded as one signal. However, since the excitation beam was scanning, the obtained fluorescence light originated from only one line scan. Thus, by simultaneously recording the data signal and the chopping square signal, it was possible to separate the data signal to two separate subsignals, one from each line scan. Each of these signals (5 KHz) was individually analyzed off line with a computer program of flow chart shown in Figure 42 to obtain the position in time and area of each peak in the signals.

The positions of the peaks from both subsignals were used to match pair of peaks, one from each line scan, that were assumed to arise from the same fluorescent molecule. Then for each pair the time difference between the positions of its two peaks were calculated. The time difference is inversely proportional to the velocity of the molecule. The average area of each pair of peaks was normalized by dividing it by the corresponding time difference. Thus, for each pair of peak, which arises from one particle, the normalized total fluorescence and the velocity were obtained.

Example 1 illustrates the method of measuring flow velocity of particles. A solution of fluorescent beads (FluoSpheres Biotin Labeled microspheres 0.2 μm, yellow green fluorescent, Molecular Probes) were introduce into the microfluidic device and their velocity were measured. The flow of fluorescent beads was achieved by capillary flow in the microfabricated channels sealed by coverslips. After a transient stage, the velocity distribution became roughly constant in time as shown in a histogram of the bead flow velocities in Figure 39. Assuming uniform distribution of beads in the channel, the theoretical probability, P_{pr}, of a particle with velocity v_0 to pass through the cross section in a given time is given by the equation:

$$P_{pr}(v = v_0) = \frac{v_0 P_v(v = v_0)}{\int v P_v dv}$$

where P_v is the probability to have a velocity v in the cross section of the channel. In circular tubes P_v is constant for $v<v_{max}$ for Poiseuille flow. The rectangular cross section of fluid flow channels of microfluidic device had an aspect (i.e., width to length) ratio of 1.3; therefore, only minor changes to P_v is introduced compare to a circular cross section fluid flow channel. Therefore, as a first order approximation the theoretical P_{pr} is proportional to v for $v<v_{max}$. This theoretical result is substantially in agreement with the experimental results. See Figure 39. The high correlation between the two graphs shows that velocity can be accurately determined using an apparatus and a method of the present invention.

In the second Example, a solution of a mix of two kinds of beads (Component A and B, LinearFlow green flow cytometry intensity calibration kit, 2.5 μm, Molecular Probes) which were similar in every other aspect besides their fluorescent intensity was introduce to the system. The fluorescent intensity of the beads was collected by the channel photomultiplier for a period of 400 sec. For each passing particle, the normalized area and velocity were calculated.

The velocity as a function of time was plotted. See Figure 38. The flow in the system is capillary flow and is driven by the pressure difference between the ends of the channel. During the time of the experiment, the pressure at the beginning of the channel, originating from the fluid at the starting basin, decreases while the pressure at the output increases. This pressure change results in a decay in the velocity over time, as shown in Figure 38 ($t > 20$sec). The abrupt change in the velocity, which occurs at about 20 sec, corresponds to the arrival of the fluid front to the output end of the fluid flow channel.

A histogram of the peak areas and the normalized peak areas was plotted. As shown in Figure 37 without normalization it is difficult to distinguish between the two kinds of beads. The coefficient of variance (CV) of the peaks were improved by the normalization from about 24% and 18% to 9.75% and 5.18% for the two kinds of beads, respectively.

An extra peak in the histogram is seen only in the graphs from the single line scan at the lower area region, which is believed to be associated with noise. Many of the noise peaks that were found in each line scans signal can not be paired with correlating peaks from the other channel. Thus, the additional low peak in the histogram is missing for the normalized areas one, which shows that the normalization method reduces noise significantly.

In a third Example, Lambda phage DNA (GIBCO) was diluted in buffer (Tris EDTA, pH 6.8 with 5 mM NaCl) and stained with the intercalating dye YOYO-1 (Molecular Probes) at a stochiometry of one dye molecule per 4 bp.

Then a solution of stained λ DNA was introduced to the microfluidic device for a period of 5 minutes. A histogram of the normalized areas of the molecules is plotted and is shown in Figure 40 The center peak in the histogram corresponds with the λ DNA (48 kbp) and the small peak in the right corresponds with hybridized pairs of λ DNA (96 kbp), i.e., λ^2. The CV for the λ and λ^2 peaks were 8.34% and 7.61%, respectively, and the ratio between the centers of the two peaks was 2.06.

While the present invention has been described herein with reference to particular embodiments thereof, a latitude of modification, various changes and substitutions are intended in the foregoing disclosure, and it will be appreciated that in some instances some features of the invention will be employed without a corresponding use of other features without departing from the scope of the invention as set forth.

Therefore, many modifications may be made to adapt a particular situation or material to the teachings of the invention without departing from the essential scope and spirit of the present invention. It is intended that the invention not be limited to the particular embodiment disclosed as the best mode contemplated for carrying out this invention, but that the invention will include all embodiments and equivalents falling within the scope of the claims.

WHAT IS CLAIMED IS:

1. A microfluidic device comprising:

a flow channel;

a pump operatively interconnected to said flow channel for moving a fluid in said flow channel; and

a damper operatively interconnected to said flow channel for reducing the fluid oscillation in said flow channel.

2. The microfluidic device of claim 1, further comprising a flow control valve operatively interconnected to said flow channel for closing and opening said flow channel.

3. The microfluidic device of claim 2, further comprising a T-junction.

4. The microfluidic device of claim 3, wherein said T-junction comprises an injection pool, a waste pool and a collection pool interconnected by said flow channel, and wherein flow channel further comprises said flow control valve proximal to said waste pool and said flow control valve proximal to said collection pool, said pump proximal to said injection pool and said damper proximal to said injection pool but posterial to said pump.

5. The microfluidic device of claim 4 further comprising a plurality of said dampers.

6. The microfluidic device of claim 1 wherein the damper comprises a cavity separated from the flow channel by a flexible membrane, the flexible membrane deflectable into the cavity to absorb energy in response to pressure oscillation within the flow channel, thereby reducing an amplitude of the pressure oscillation.

7. The microfluidic device of claim 6 further comprising a constriction in a width of the flow channel positioned downstream of the flexible membrane.

8. The microfluidic device of claim 1 wherein the damper comprises an enlarged portion of the flow channel partially filled with a fluid, the fluid compressible

to absorb energy in response to pressure oscillation within the flow channel, thereby reducing an amplitude of the pressure oscillation.

9. The microfluidic device of claim 1 wherein the damper comprises elastomeric material forming walls of the flow channel, the elastomeric material deflectable to absorb energy in response to pressure oscillation within the flow channel, thereby reducing an amplitude of the pressure oscillation.

10. A microfluidic sorting device comprising:

a first flow channel formed in a first layer of elastomer material, a first end of the first flow channel in fluid communication with a collection pool and a second end of the first flow channel in fluid communication with a waste pool;

a second flow channel formed in the first elastomer layer, a first end of the second flow channel in fluid communication with an injection pool and a second end of the second flow channel in fluid communication with the first flow channel at a junction;

a collection valve adjacent to a first side of the junction proximate to the collection pool, the collection valve comprising a first recess formed in a second elastomer layer overlying the first elastomer layer, the first recess separated from the first flow channel by a first membrane portion of the second elastomer layer deflectable into the first flow channel;

a waste valve adjacent to a second side of the junction proximate to the waste pool, the waste valve comprising a second recess formed in the second elastomer layer separated from the second flow channel by a second membrane portion of the second elastomer layer deflectable into the first flow channel;

a pump adjacent to a third side of the junction proximate to the injection pool, the pump comprising at least pressure channels formed in the second elastomer layer and separated from second flow channel by third membrane portions of the second elastomer layer deflectable into the second flow channel; and

a detection region positioned between the injection pool and the junction, one of an open and closed state of the collection valve and the waste valve determined by an identity of a sortable entity detected in the detection region.

11. The microfluidic device of claim 10 further comprising a damper structure adjacent to the second flow channel between the pump and the detection region.

12. The microfluidic device of claim 11 wherein the damper comprises a cavity formed in the second elastomer layer and separated from the second flow channel by a flexible membrane, the flexible membrane deflectable into the cavity to absorb energy in response to pressure oscillation within the second flow channel, thereby reducing an amplitude of the pressure oscillation.

13. The microfluidic device of claim 12 further comprising a constriction in a width of the second flow channel between the flexible membrane and the junction.

14. The microfluidic device of claim 11 wherein the damper comprises an enlarged portion of the flow channel partially filled with a fluid, the fluid compressible to absorb energy in response to pressure oscillation within the flow channel, thereby reducing an amplitude of the pressure oscillation.

15. The microfluidic device of claim 10 wherein the elastomer material forming walls of the first flow channel is deflectable to absorb energy in response to pressure oscillation within the flow channel, thereby reducing an amplitude of the pressure oscillation.

16. The microfluidic device of claim 10 wherein the junction is T-shaped.

17. The microfluidic device of claim 10 further comprising a second sorter structure positioned between the waste valve and the waste pool.

18. A damper for a microfluidic device comprising:
a flow channel formed in an elastomer material; and
an energy absorber adjacent to the flow channel and configured to absorb an energy of oscillation of a fluid positioned within the flow channel.

19. The damper of claim 18 wherein the energy absorber comprises a flexible elastomer membrane positioned between the flow channel and a cavity, the flexible membrane deflectable into the cavity to absorb the energy of oscillation in the flow channel.

20. The damper of claim 18 wherein the energy absorber comprises a fluid positioned within an enlarged portion of the flow channel, the fluid compressible to absorb the energy of oscillation.

21. The damper of claim 18 wherein the energy absorber comprises elastomer material on the side walls of the flow channel, the elastomer material deflectable to absorb the energy of oscillation.

22. The damper of claims 19, 20, or 21 further comprising a constriction in a width of the flow channel downstream of the energy absorber.

23. A sorting method comprising:

deflecting a first elastomer membrane of an elastomer block into a flow channel to cause a sortable entity to flow into a detection region positioned upstream of a junction in the flow channel;

interrogating the detection region to identify the sortable entity within the detection region;

based upon an identity of the sortable entity, deflecting one of a second membrane and a third membrane of the elastomer block into one of a first branch flow channel portion and a second branch flow channel portion located downstream of the junction to cause the sortable entity to flow to one of a collection pool or a waste pool.

24. The method of claim 23 wherein the sortable entity is flowed once through the detection region.

25. The method of claim 23 wherein:

the sortable entity is initially flowed through the detection region; and then

a direction of flow is reversed to flow the sortable entity back into the detection region.

26. The method of claim 23 further comprising dampening an oscillation of energy within the flow channel upstream of the junction.

27. A method for sorting a material using a microfluidic device of claim 4.

28. The method of claim 27 comprising using a reversible sorting process.

29. A method for dampening pressure oscillations in a flow channel comprising providing an energy absorber adjacent to the flow channel, such that the energy absorber experiences a change in response the pressure oscillations.

30. The method of claim 29 wherein the energy absorber comprises a flexible membrane that deflects into a cavity in response to the pressure oscillations.

31. The method of claim 29 wherein the energy absorber comprises a fluid pocket that experiences compression in response to the pressure oscillations.

32. The method of claim 29 wherein the energy absorber comprises an elastomeric flow channel sidewall that experiences deformation in response to the pressure oscillations.

33. The method of claim 29 further comprising constricting a width of the flow channel downstream of the energy absorber.

34. A method for determining a velocity independent characteristic parameter of an analyte, wherein the characteristic parameter of the analyte is capable of being influenced by or dependent on the velocity of the analyte, said method comprising:

(a) providing a means for transporting a fluid medium comprising the analyte from a first position to a second position of a fluid flow channel of a fluidic device;

(b) measuring the characteristic parameter of the analyte within the fluid flow channel at a plurality of locations along the fluid flow channel in between the first and the second position; and

(c) determining the velocity independent characteristic parameter of the analyte using the measured characteristic parameters of step (b) and normalizing the measurement by substantially eliminating the velocity component of the measurement.

35. The method of Claim 34, wherein said method comprises velocity independent flow cytometry.

36. The method of Claim 34, wherein said fluidic device is a microfluidic device.

37. The method of Claim 36, wherein said means for transporting a fluid medium comprises a peristaltic pump or electroosmosis.

38. The method of Claim 36, wherein the substantially all analytes passes through each detection zone individually.

39. The method of Claim 38, wherein said step (b) for measuring the characteristic parameter of the analyte comprises a detector comprising a laser, a laser beam guiding device, and a means for detecting laser induced fluorescence.

40. The method of Claim 39, wherein said laser beam guiding device is an acousto-optic modulator.

41. The method of Claim 38, wherein said step (c) of determining the velocity independent characteristic parameter of the analyte comprises:

(i) comparing signals obtained from the plurality of locations along the fluid flow channel in step (b);

(ii) determining a time difference by calculating the time it takes for a particular analyte to pass from a first detection position to a second detection position; and

(iii) determining the velocity independent characteristic parameter using the time difference.

42. The method of Claim 41, wherein said step (iii) of determining velocity independent characteristic parameter comprises averaging the signal of the particle from the first and the second detection zone and normalizing the average signal using the time difference.

43. The method of Claim 42, wherein the analyte is a cell, an oligonucleotide or an organic compound.

44. The method of Claim 43, wherein the analyte is a cell and said method is used for cell sorting.

45. The method of Claim 43, wherein the analyte is an oligonucleotide and said method is used determine the number of nucleotides in the oligonucleotide.

46. The method of Claim 45, wherein said step of determining number of nucleotides in the oligonucleotide comprises:

(A) attaching a fluorescent molecule to the oligonucleotide to produce a modified oligonucleotide prior to measuring velocity independent characteristic parameter of the modified oligonucleotide, wherein said characteristic parameter is integrated fluorescent peak area of said modified oligonucleotide; and

(B) determining the number of nucleotides in the oligonucleotide by comparing the velocity independent integrated fluorescence peak area of the modified oligonucleotide with a velocity independent fluorescence peak area of a standard oligonucleotide, wherein the velocity independent fluorescence peak area of the standard oligonucleotide has been calibrated to the number of nucleotides present the standard oligonucleotide.

47. An apparatus for determining a characteristic parameter of an analyte in a fluid medium independent of the flow velocity of the analyte, said apparatus comprising:

(a) a device comprising a fluid flow channel;

(b) a means for transporting a fluid medium from a first position to a second position of the fluid flow channel;

(c) a plurality of detection zones located at different positions along said fluid flow channel and located in between the first and the second position of the fluid flow channel;

(d) a detector for detecting the analyte flowing through the detection zone; and

(e) a means for measuring a characteristic parameter of the analyte independent of the flow velocity of the analyte through the plurality of detection zones.

48. The apparatus according to Claim 47, wherein said device is a microfluidic device.

49. The apparatus according to Claim 48, wherein said means for transporting a fluid medium comprises a peristaltic pump or electroosmosis.

50. The apparatus according to Claim 49 wherein said detector comprises a laser, an acousto-optic modulator and a fluorescence measuring device.

51 The apparatus according to Claim 50 wherein said analyte is selected from the group consisting of a cell and an oligonucleotide.

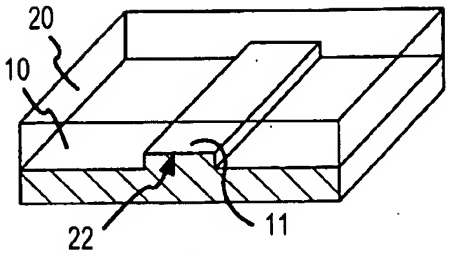


FIG.1

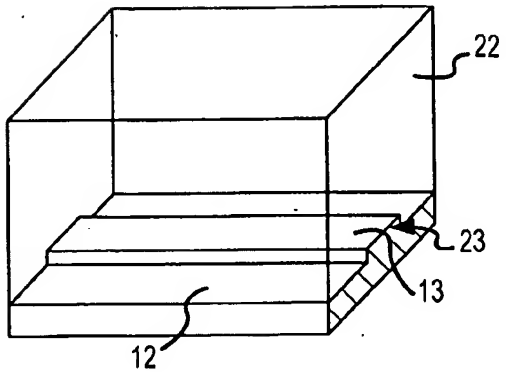


FIG.2

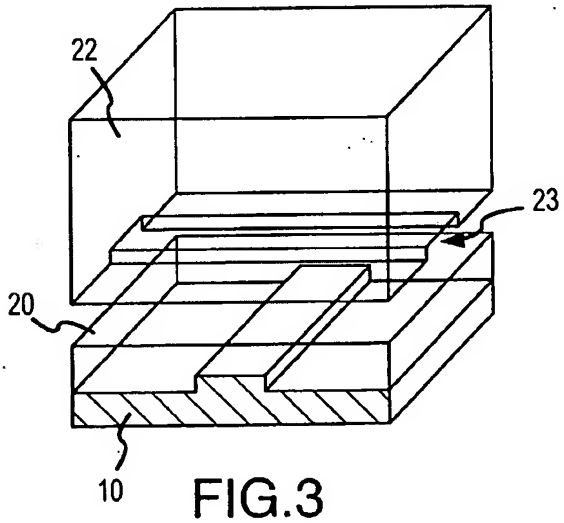


FIG.3

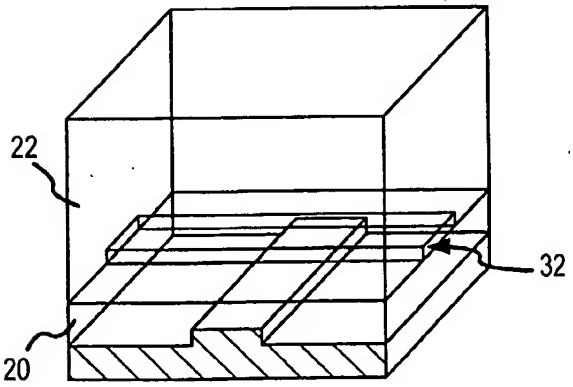


FIG.4

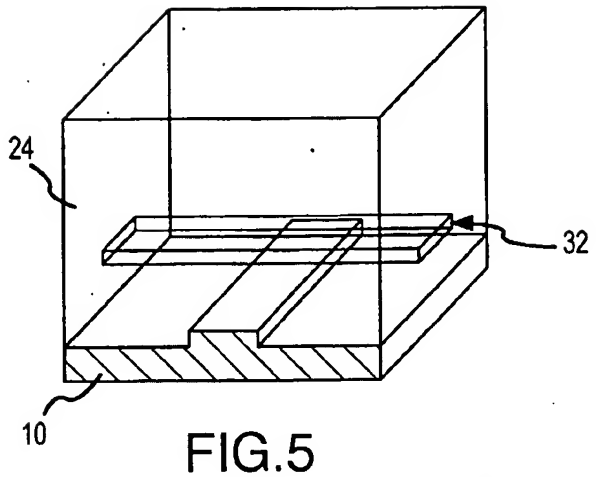


FIG.5

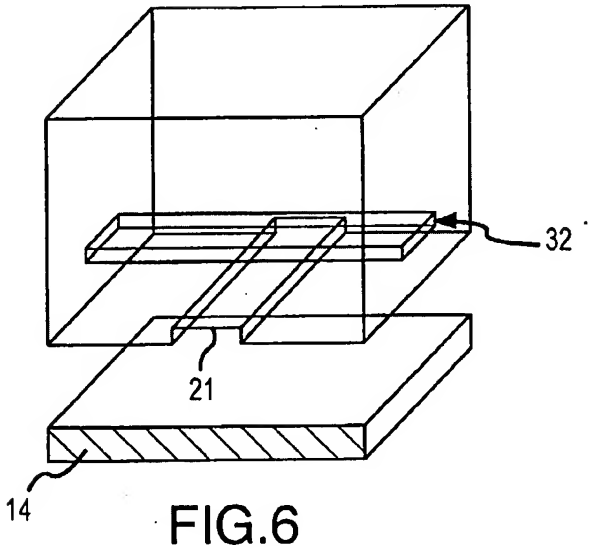


FIG.6

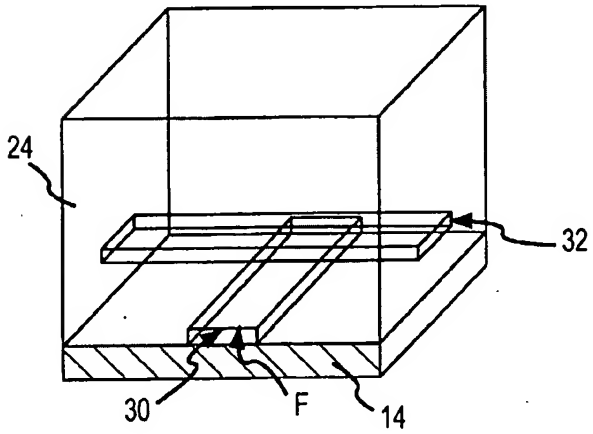


FIG.7A

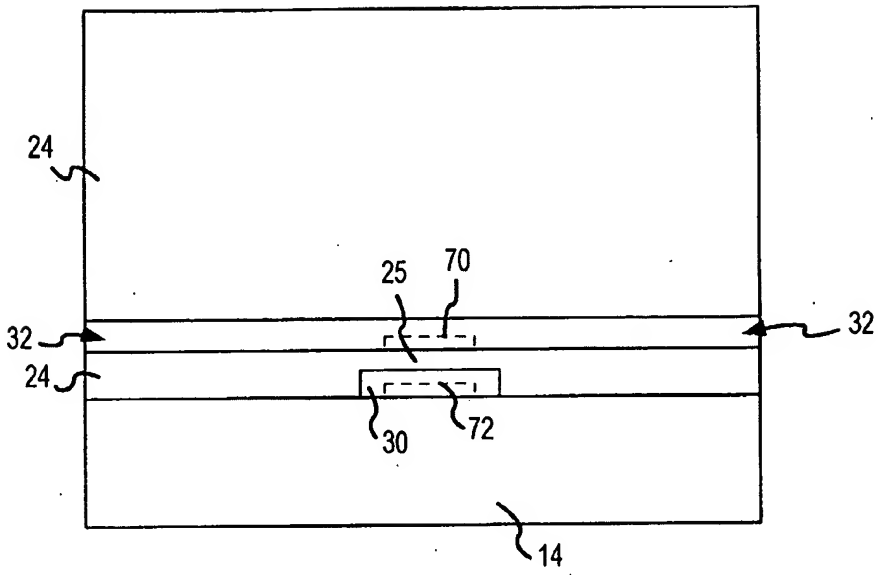


FIG.7B

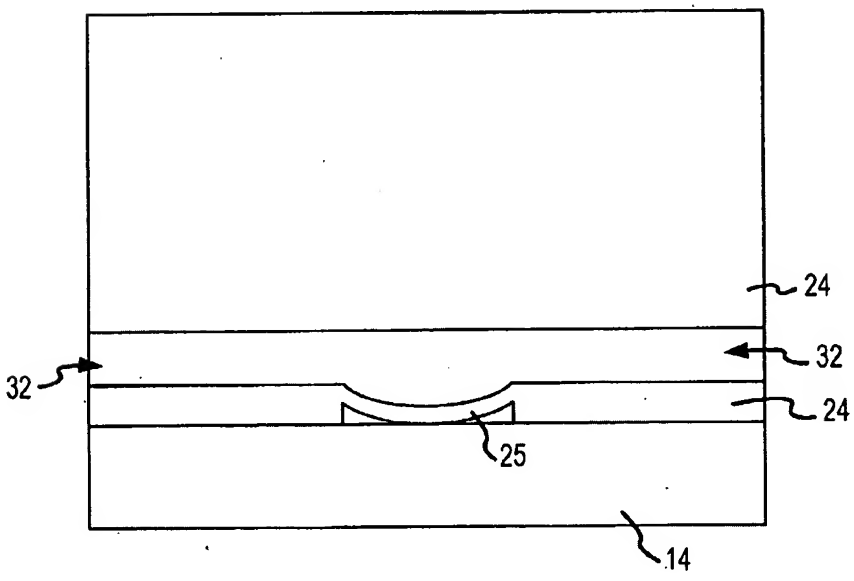


FIG.7H

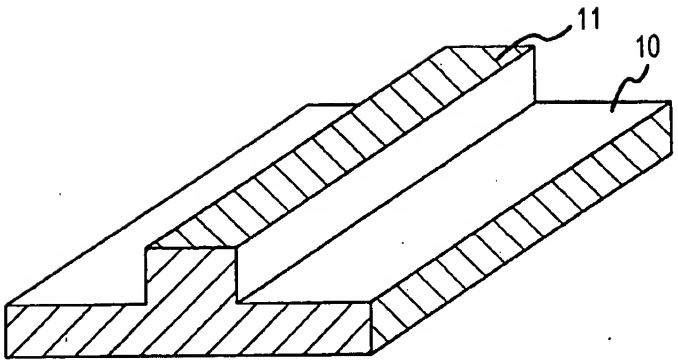


FIG.7C

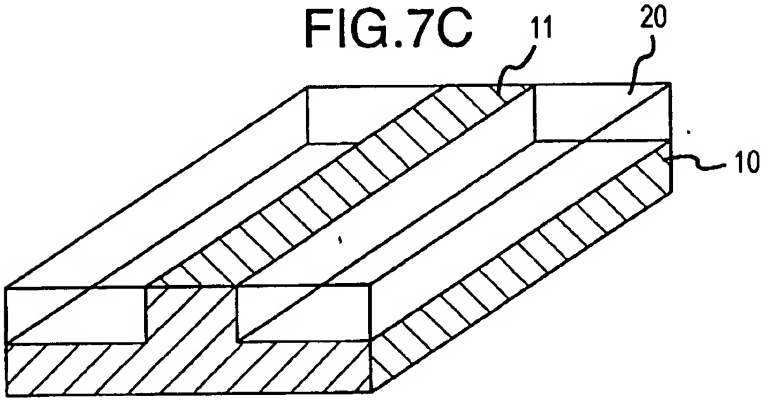


FIG.7D

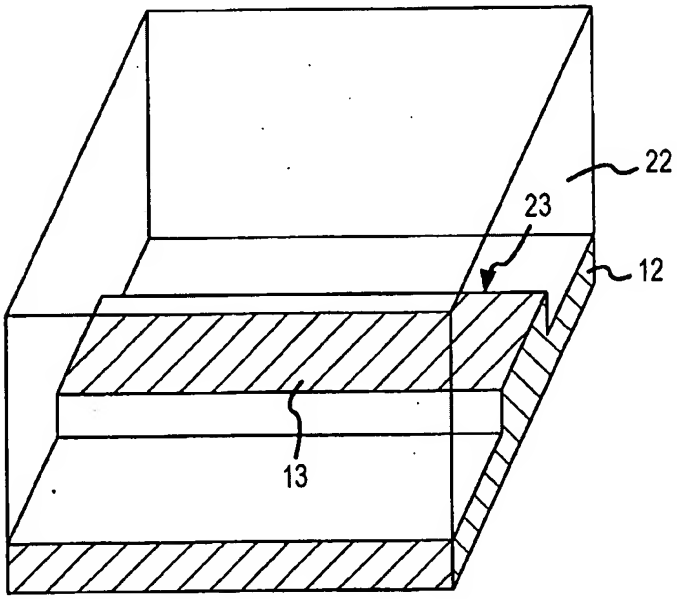


FIG.7E

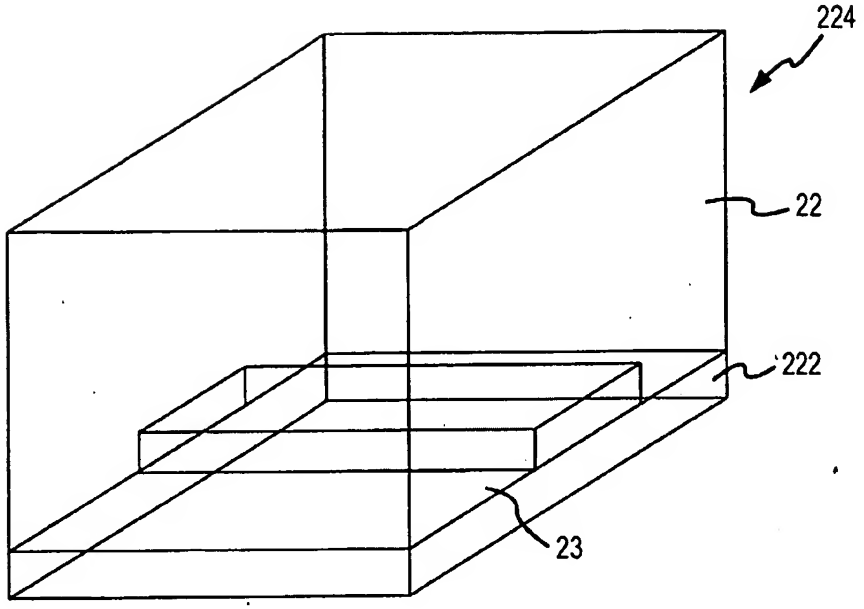


FIG.7F

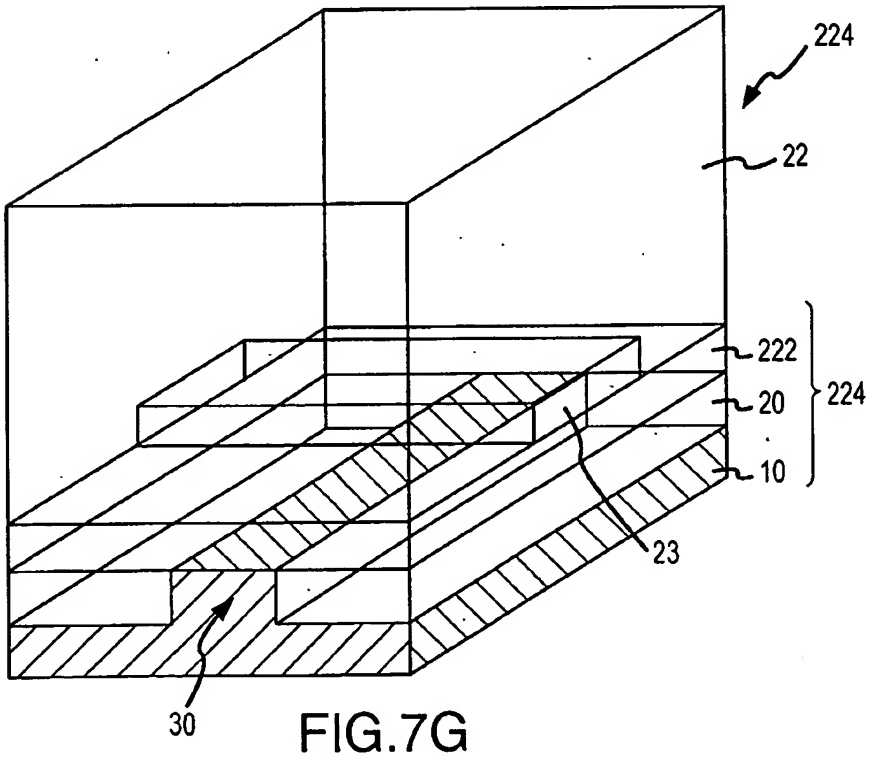


FIG.7G

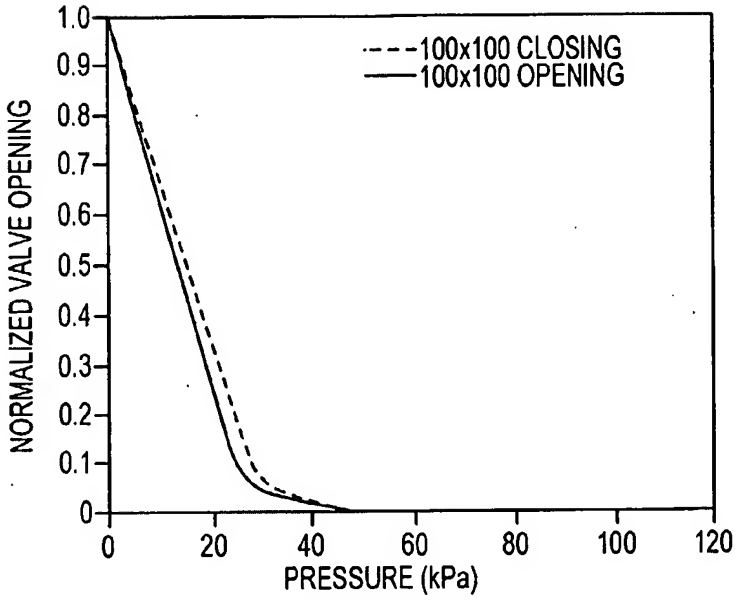

100x100 CLOSING
100x100 OPENING
NORMALIZED VALVE OPENING
PRESSURE (kPa)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
20
40
60
80
100
120

FIG.8A

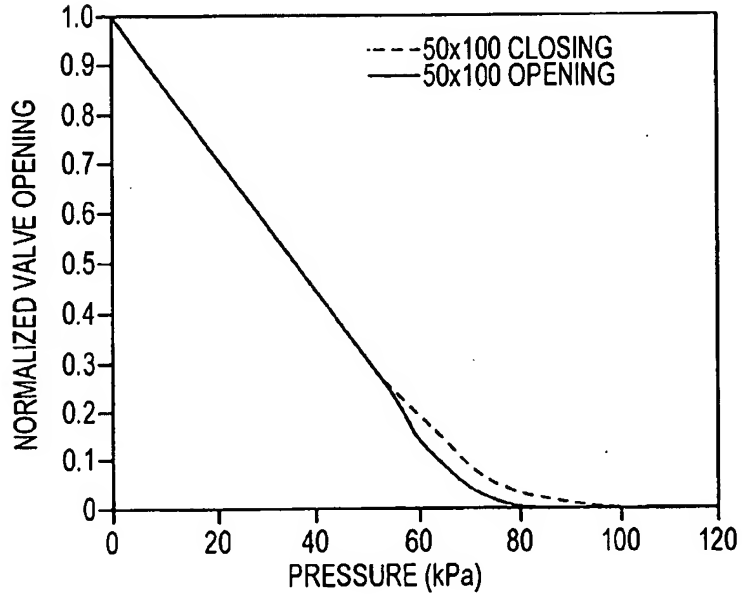
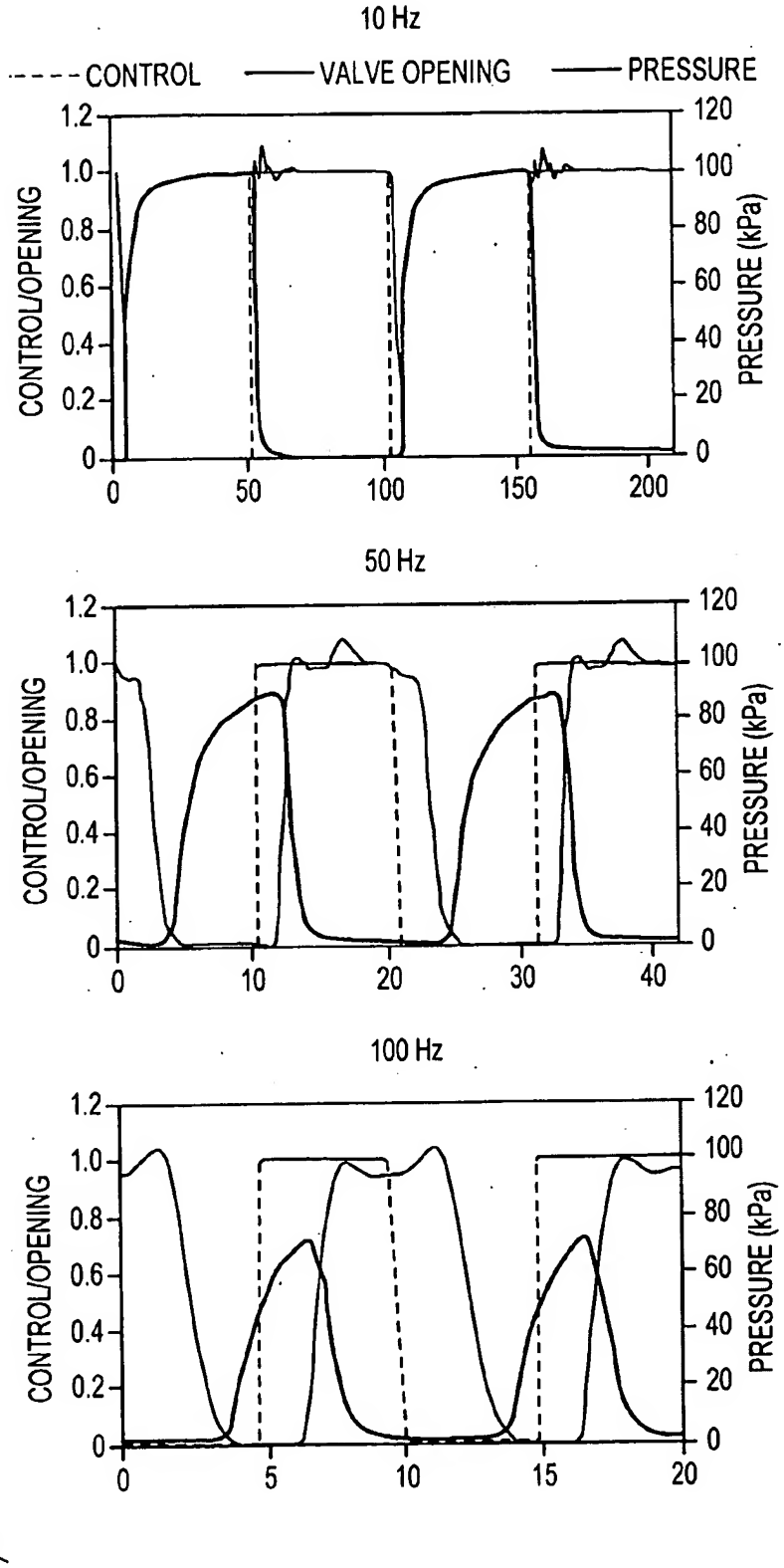

50x100 CLOSING
50x100 OPENING
NORMALIZED VALVE OPENING
PRESSURE (kPa)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
20
40
60
80
100
120

FIG.8B

FIG.9


10 Hz
CONTROL
VALVE OPENING
PRESSURE
CONTROL/OPENING
PRESSURE (kPa)
50 Hz
CONTROL/OPENING
PRESSURE (kPa)
100 Hz
CONTROL/OPENING
PRESSURE (kPa)

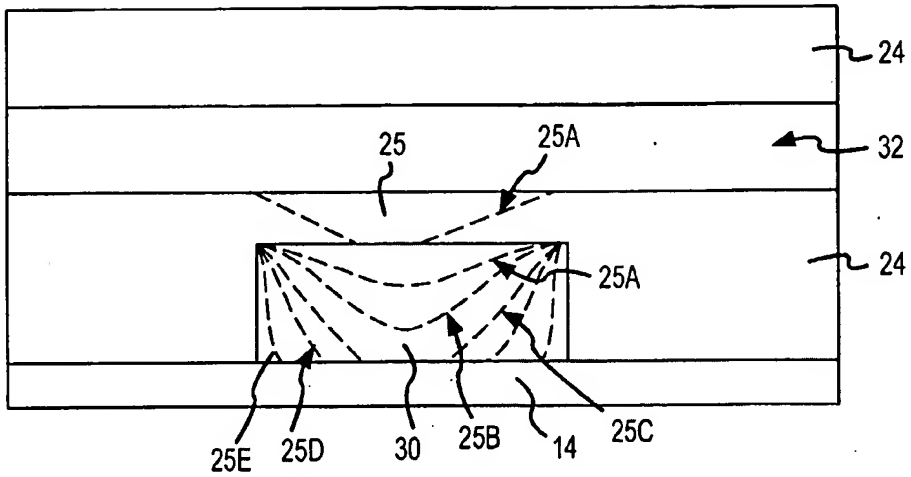

24
32
25
25A
24
25A
25E
25D
30
25B
14
25C

FIG.10

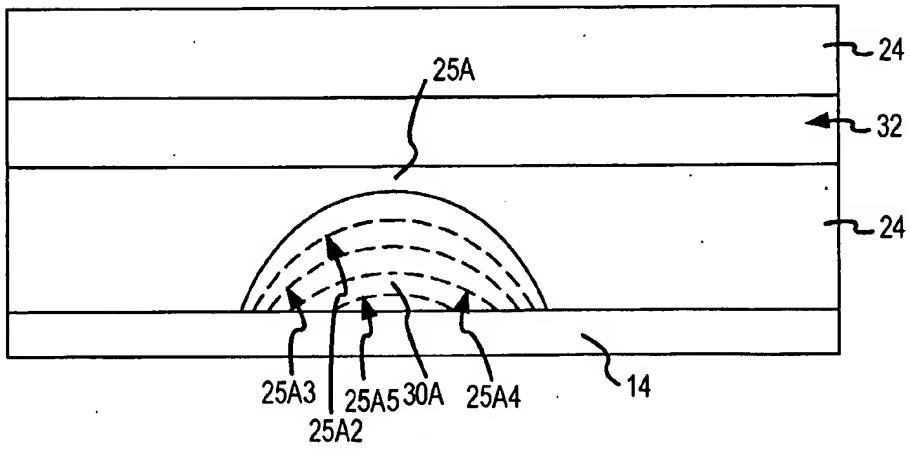

24
25A
32
24
25A3
25A2
25A5
30A
25A4
14

FIG.11

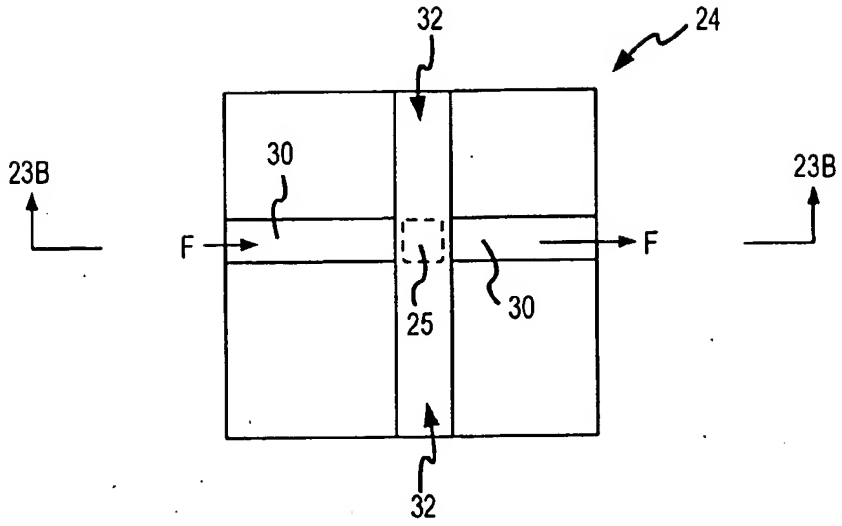


FIG.12A

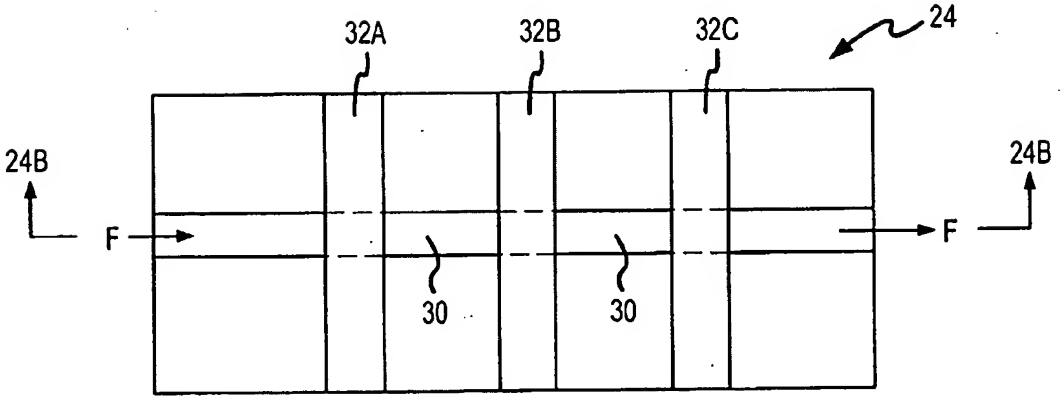


FIG.13A

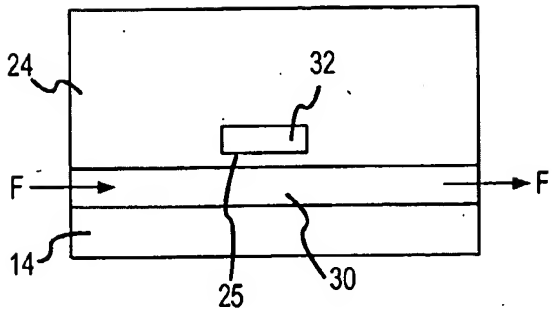


FIG.12B

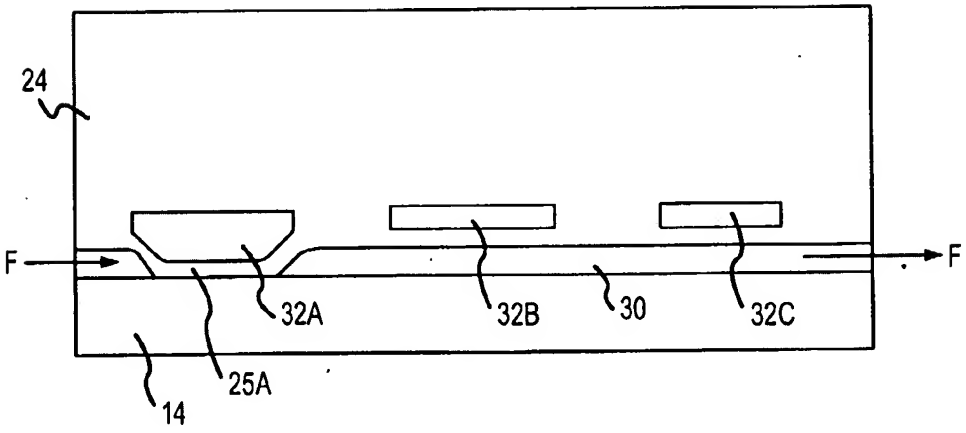


FIG.13B

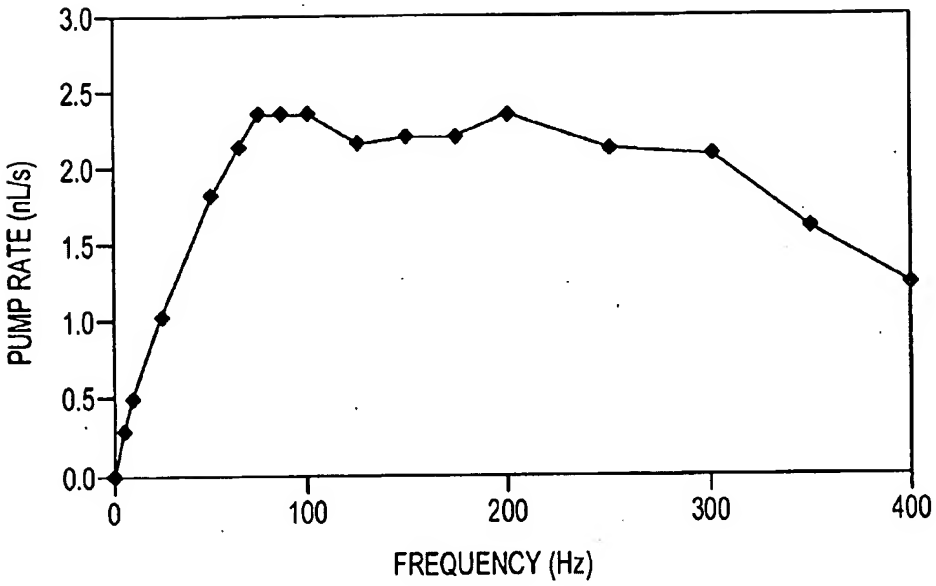


FIG.14

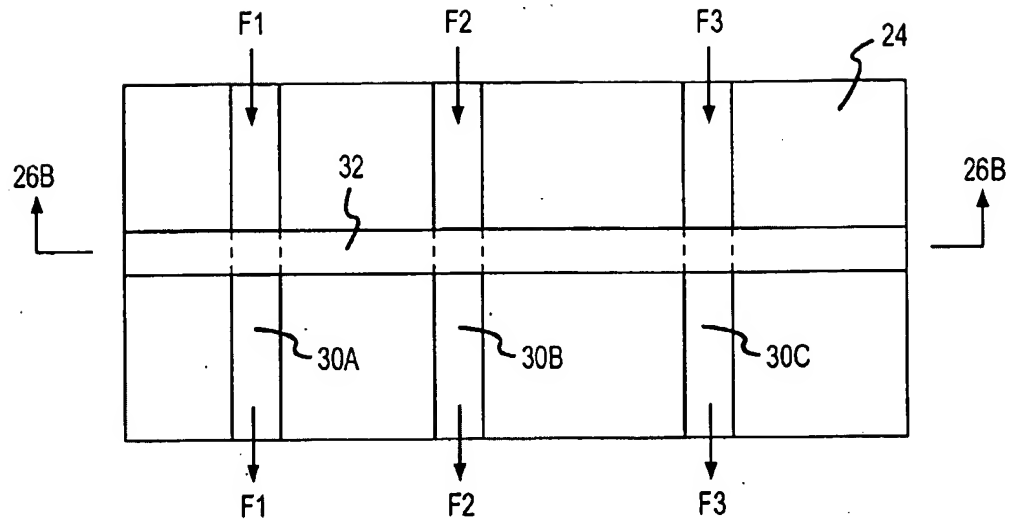


FIG.15A

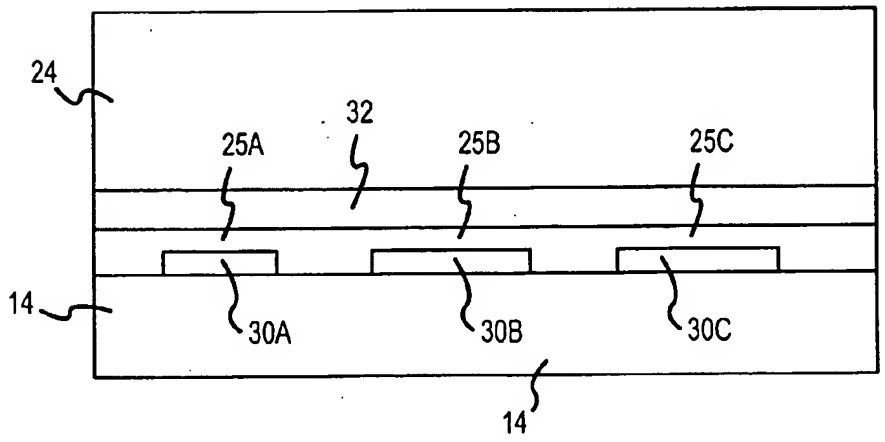


FIG.15B

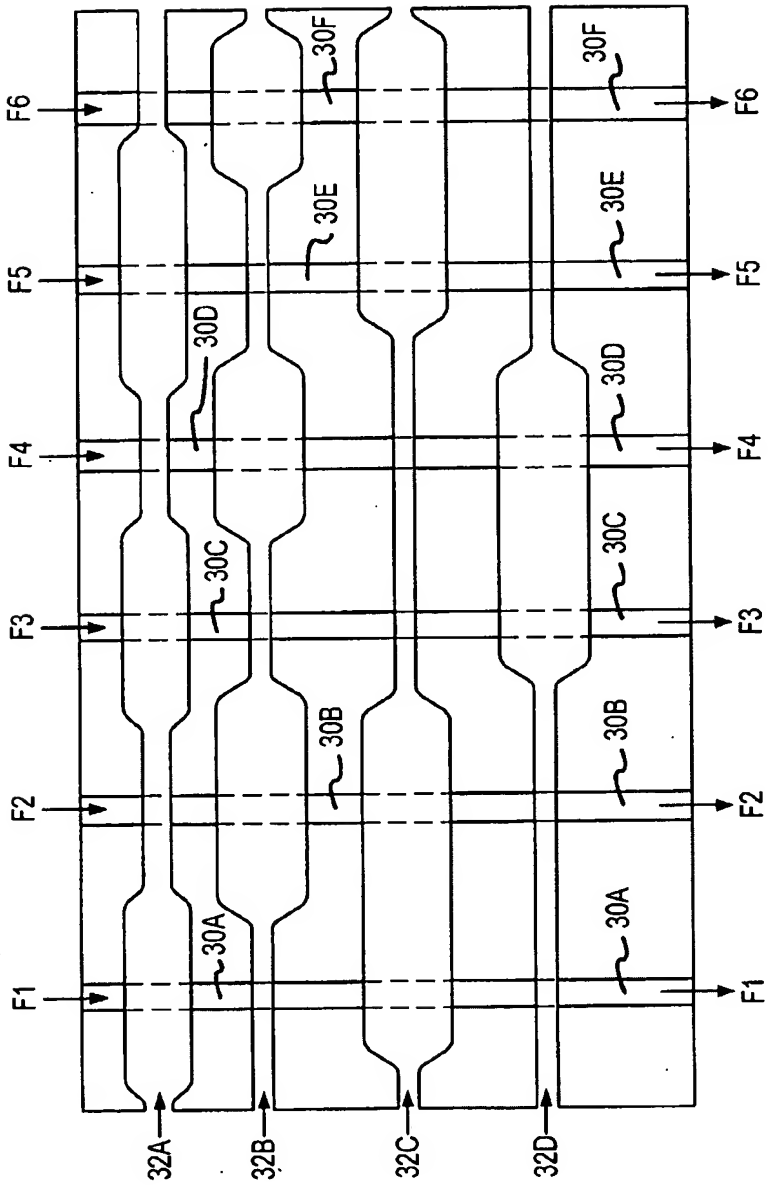


FIG.16

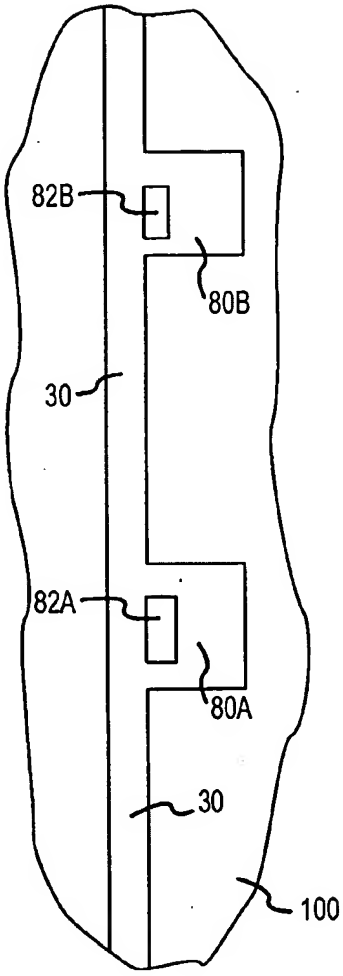


FIG.17A

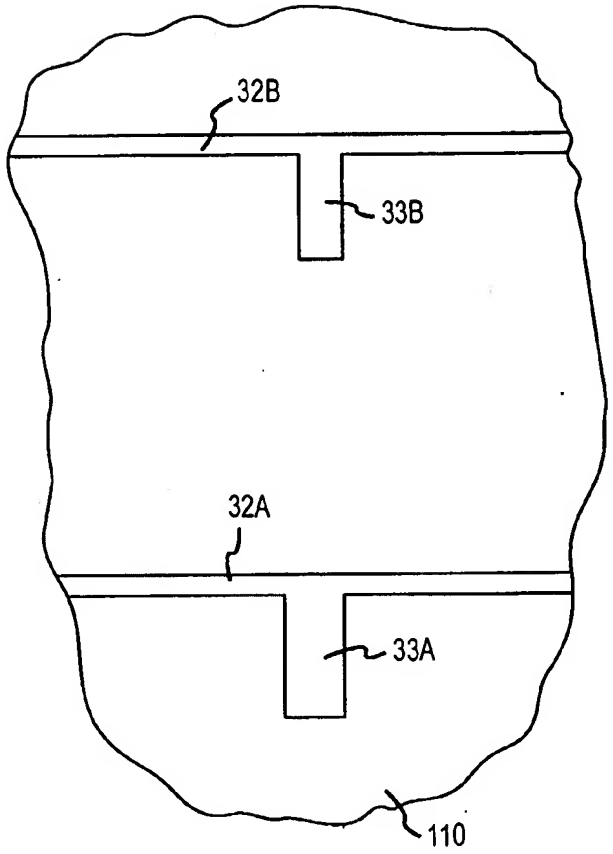


FIG.17B

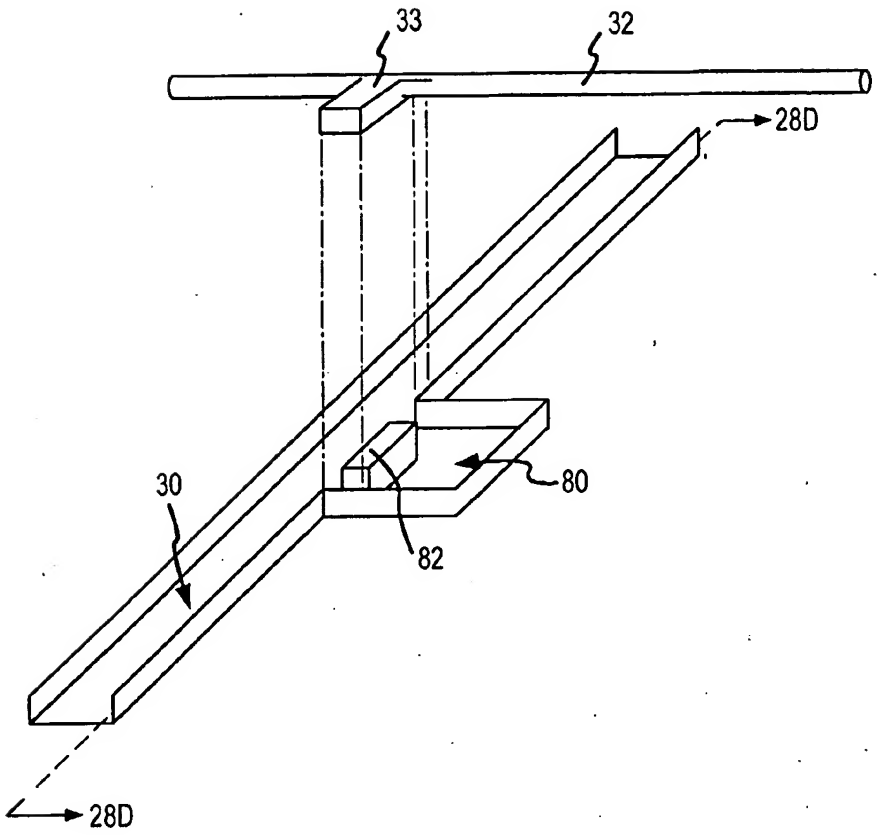


FIG.17C

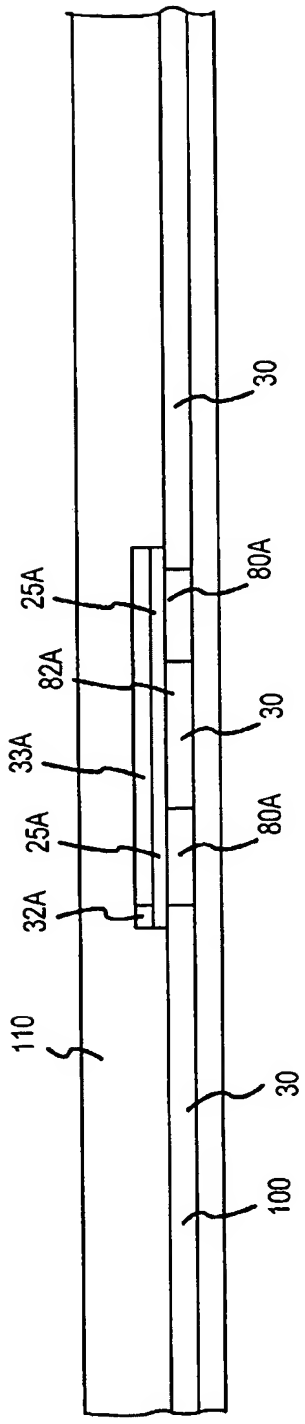


FIG.17D

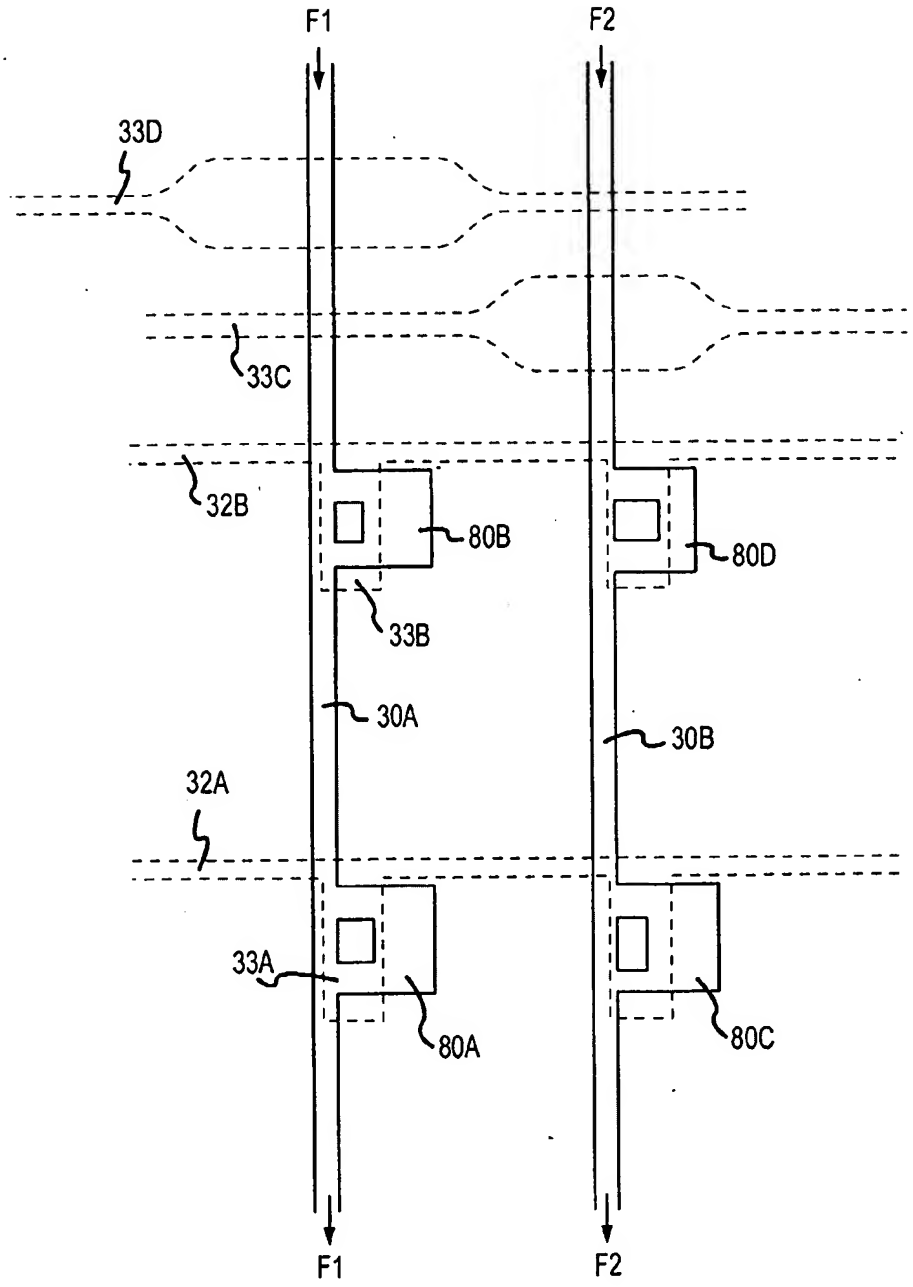


FIG.18

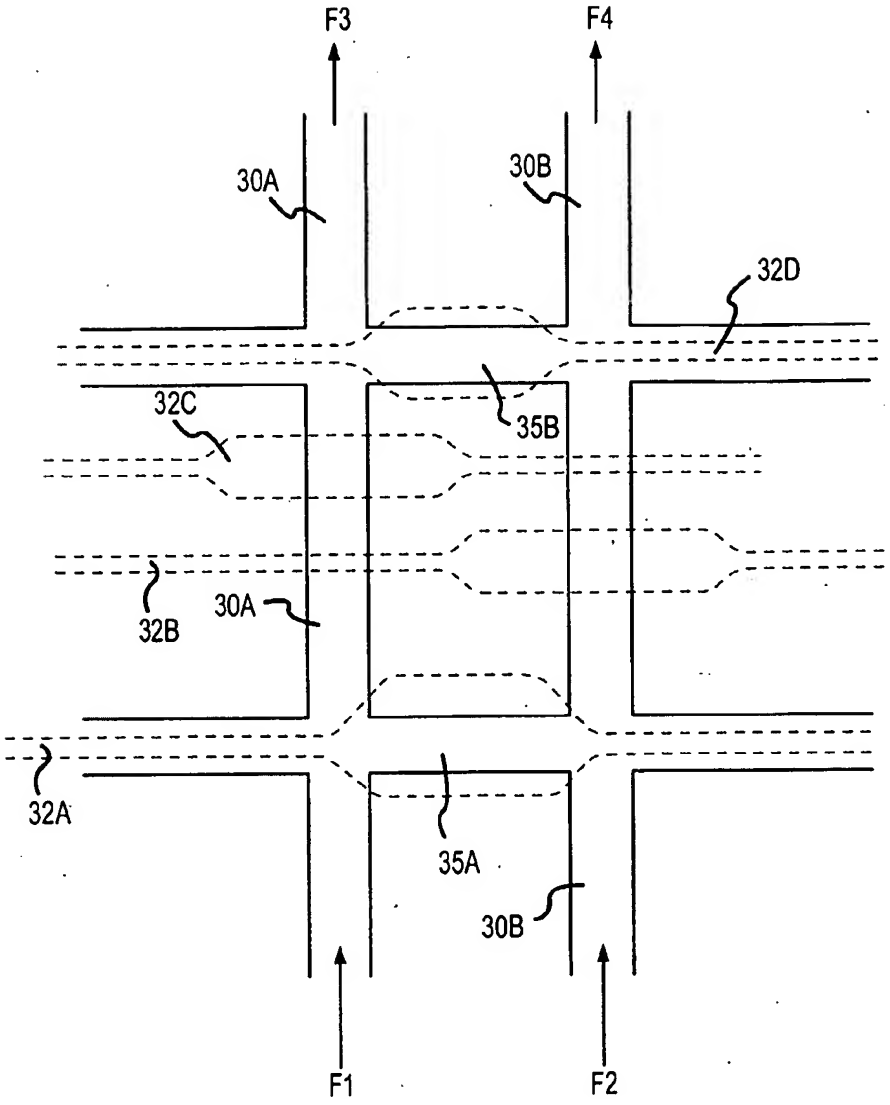


FIG.19

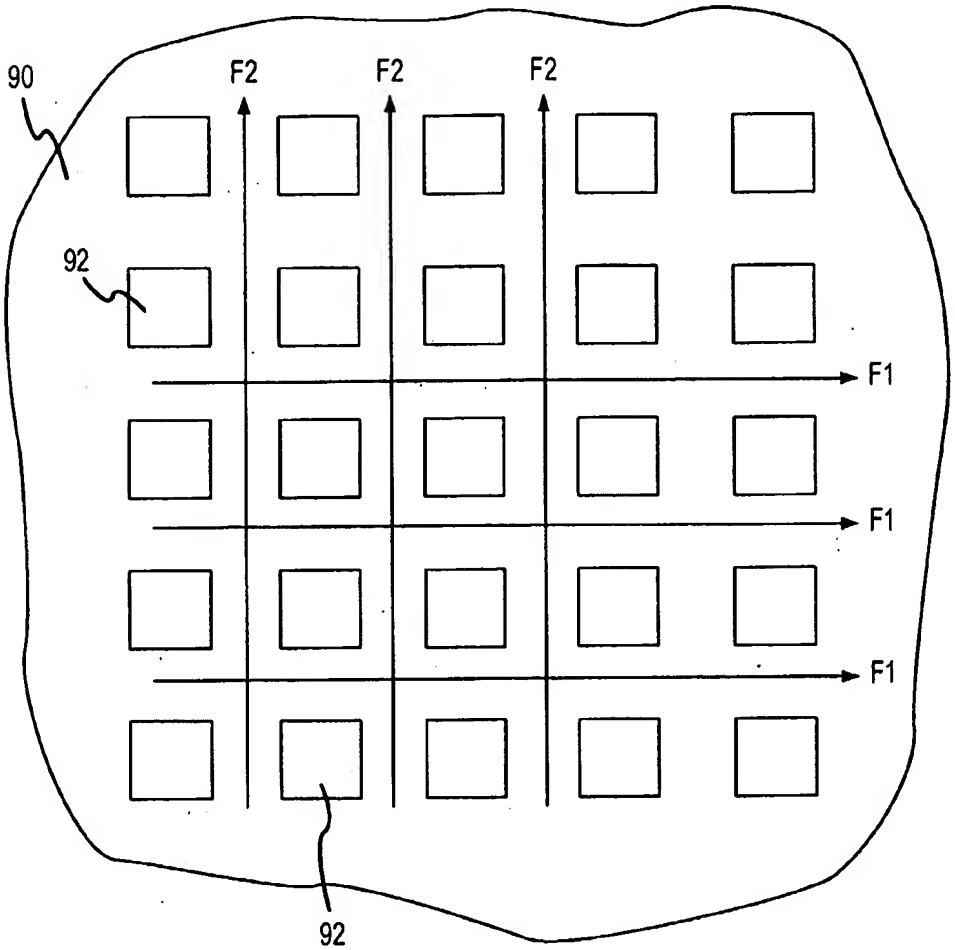


FIG.20A

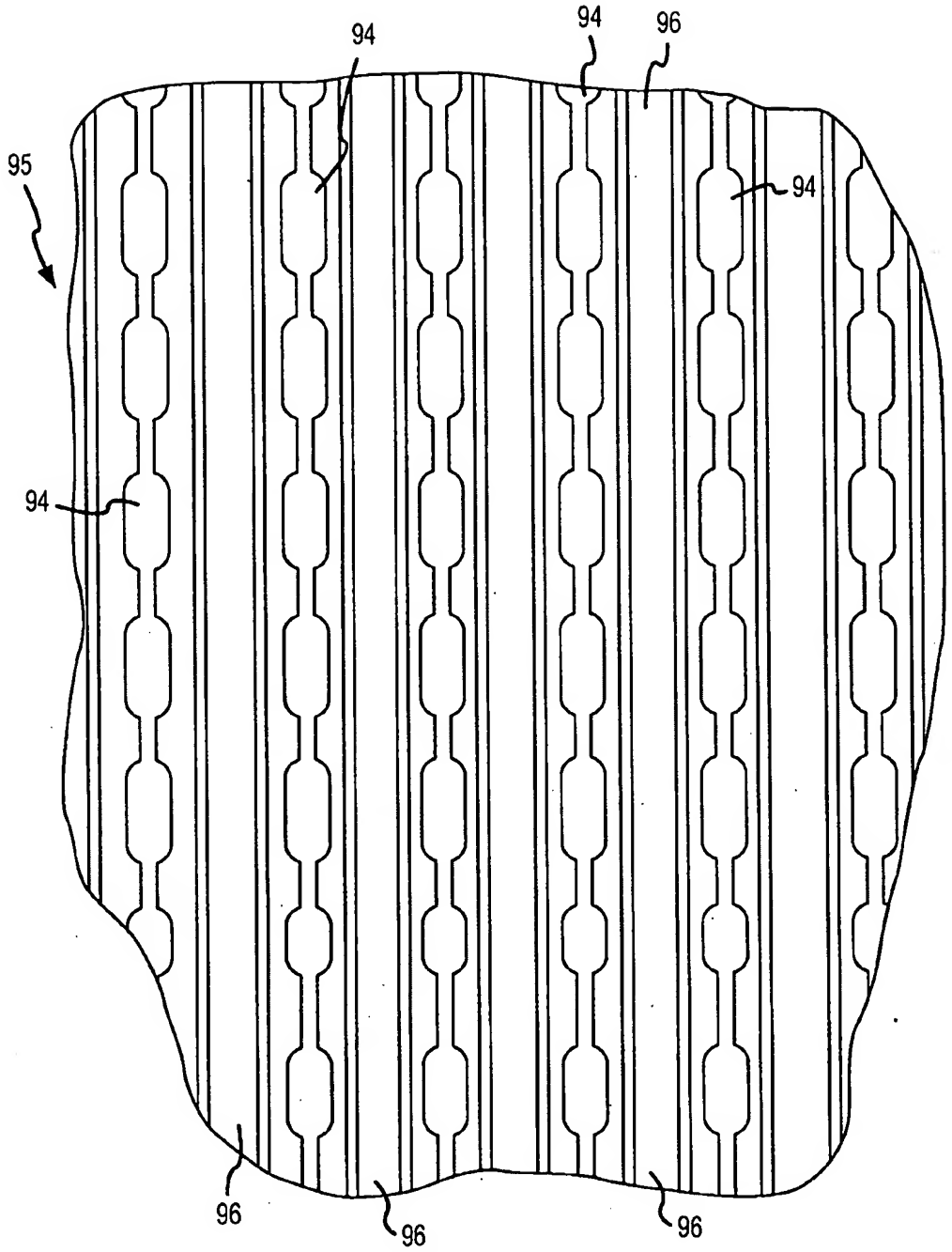


FIG.20B

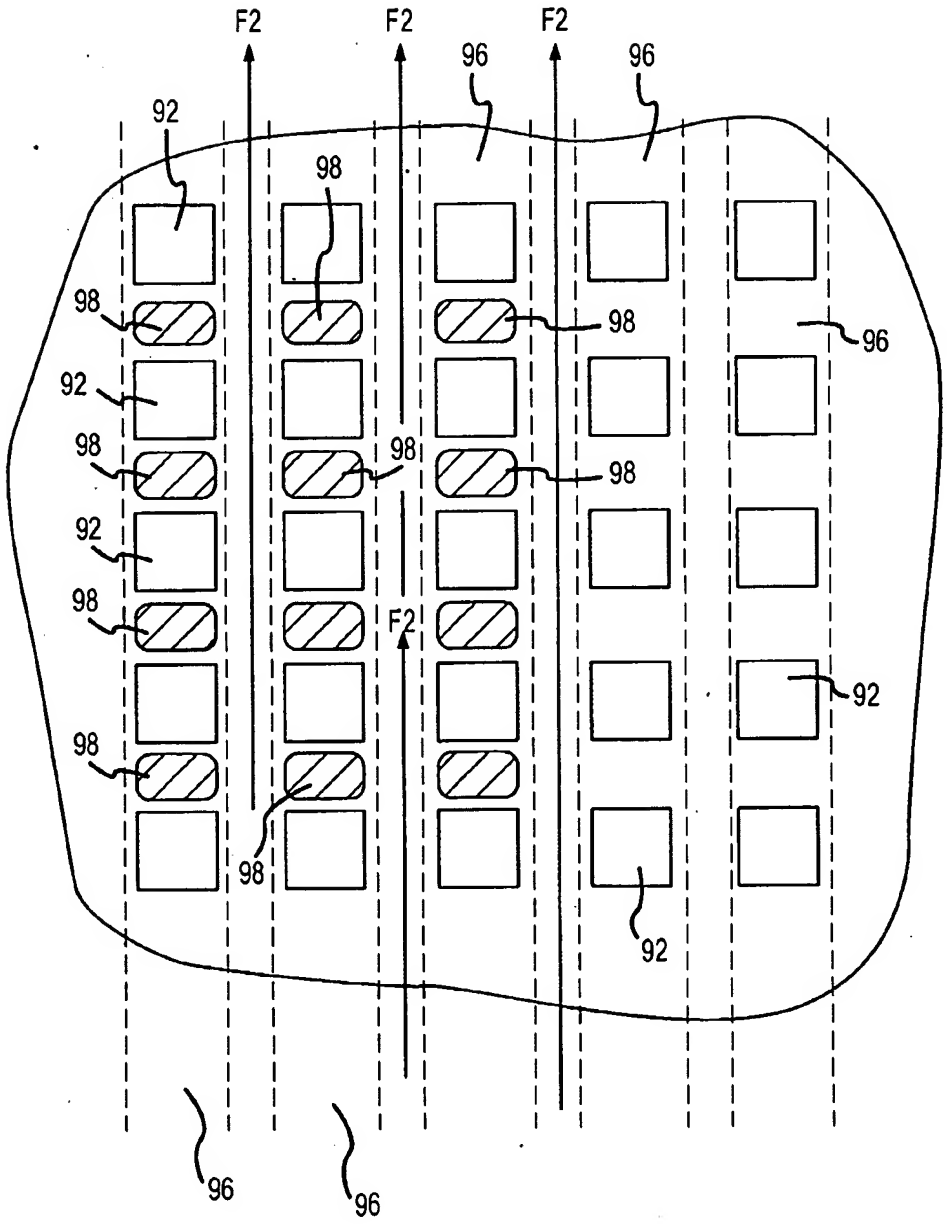


FIG.20C

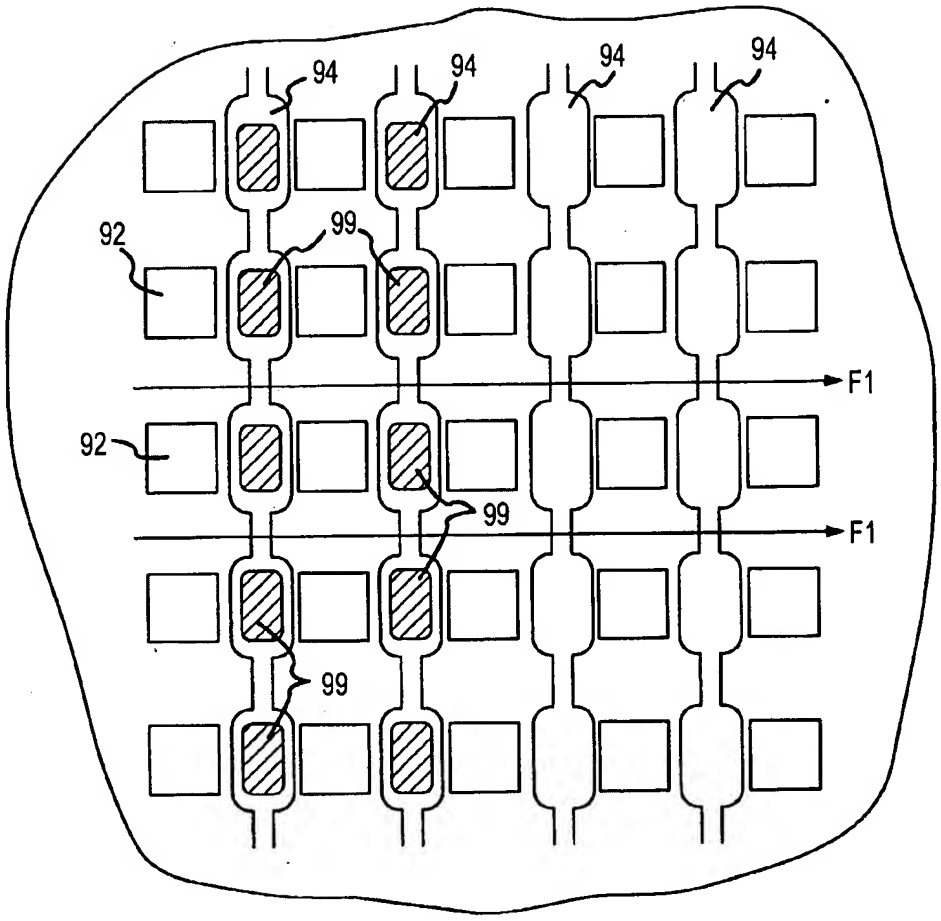


FIG.20D

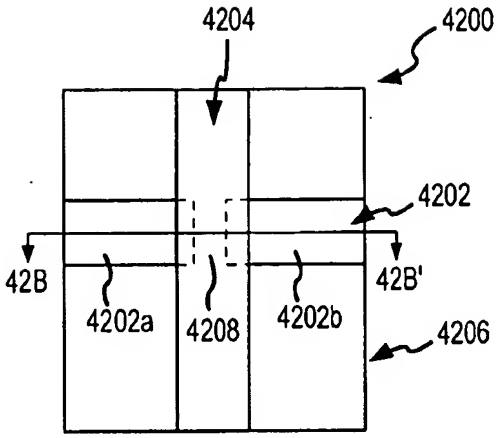


FIG.21A

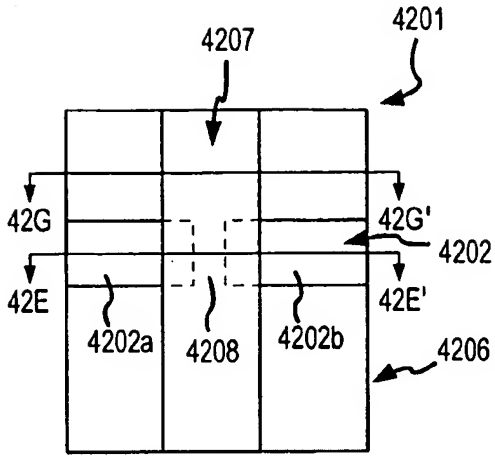


FIG.21D

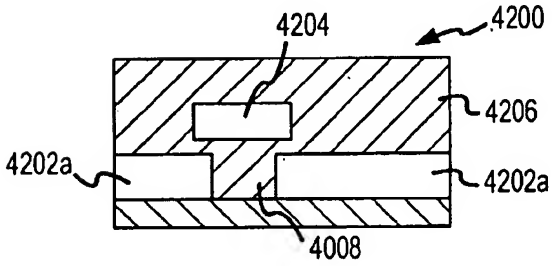


FIG.21B

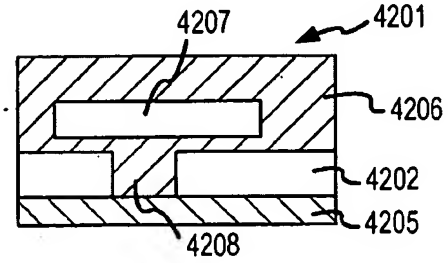


FIG.21E

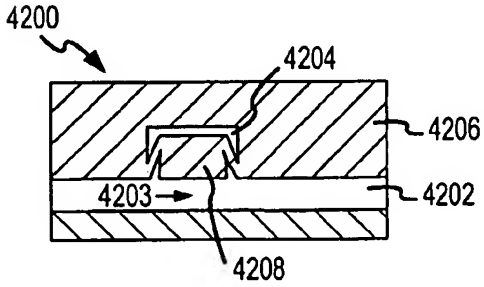


FIG.21C

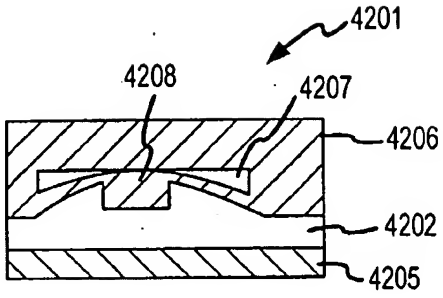


FIG.21F

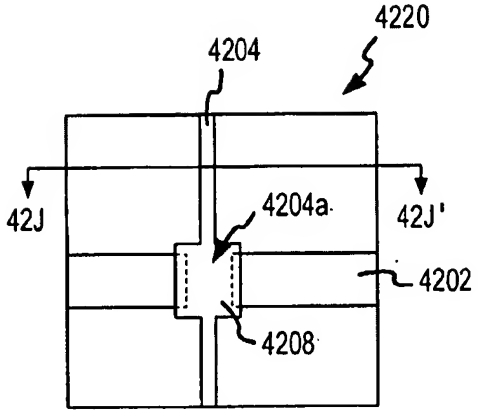


FIG.21I

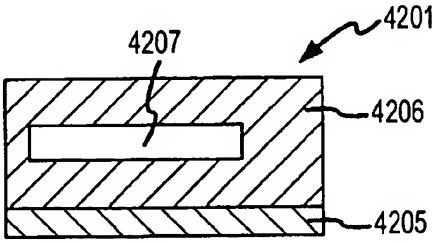


FIG.21G

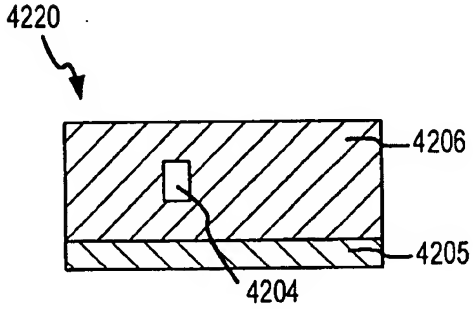


FIG.21J

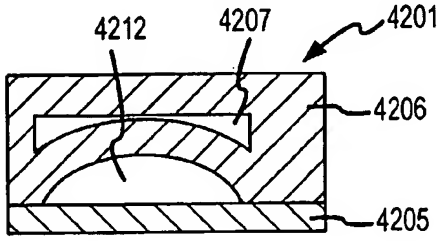


FIG.21H

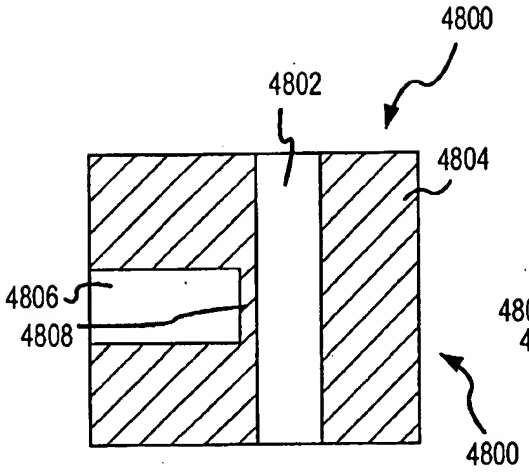


FIG.22A

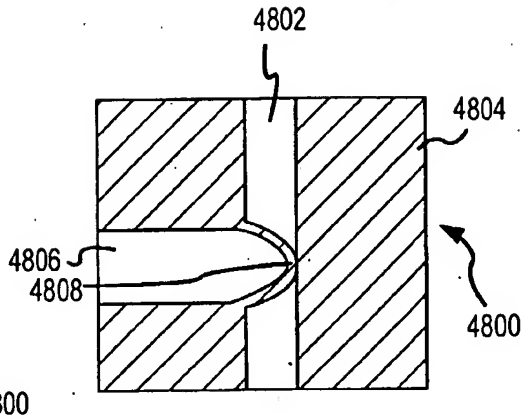


FIG.22B

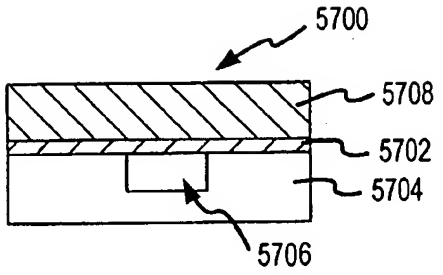


FIG.23

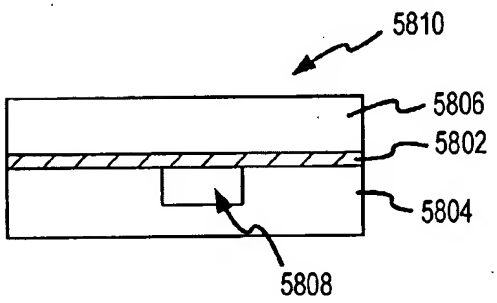


FIG.24

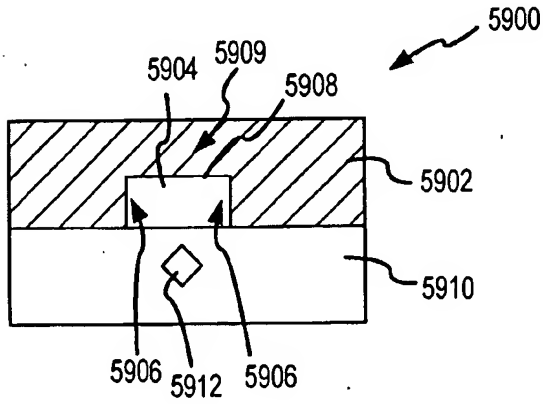


FIG.25

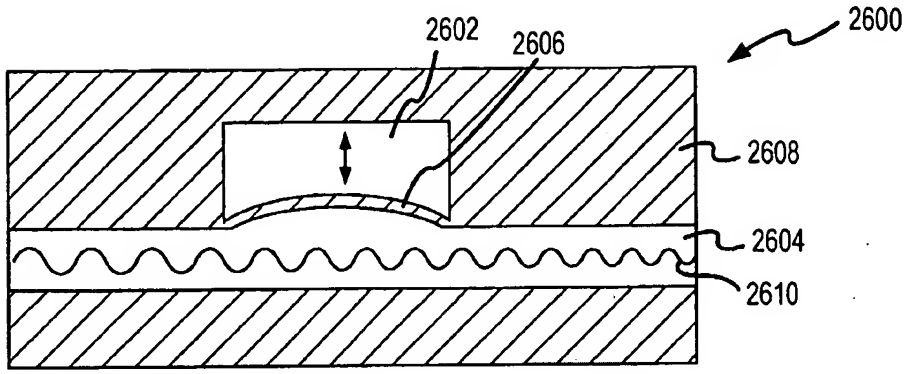


FIG.26

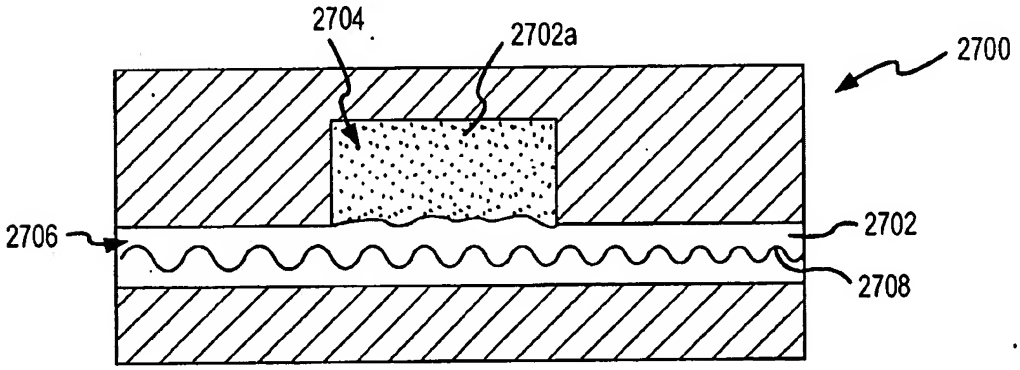


FIG.27

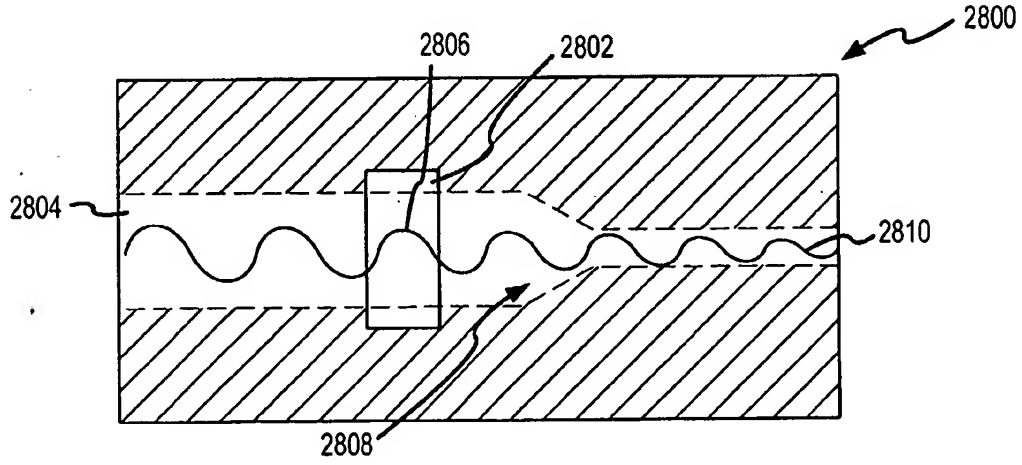


FIG.28

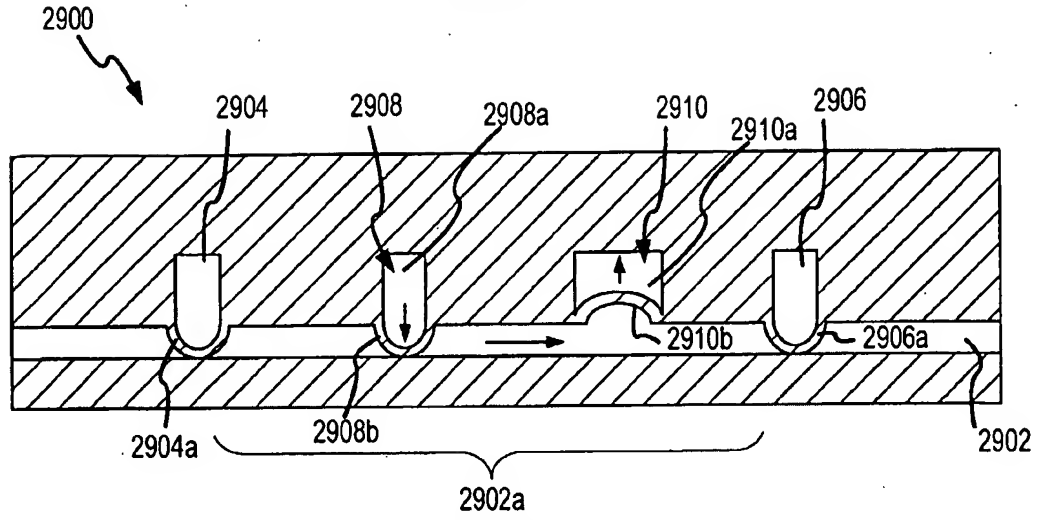


FIG.29A

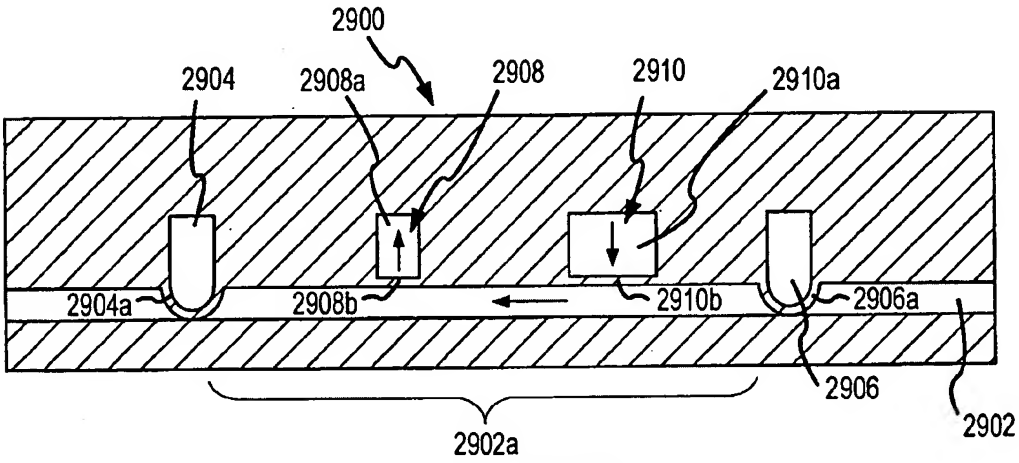


FIG.29B

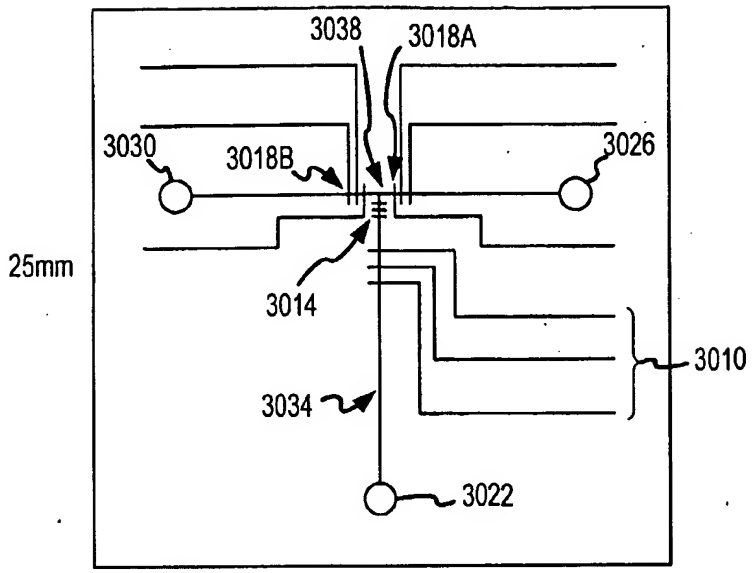


FIG.30A

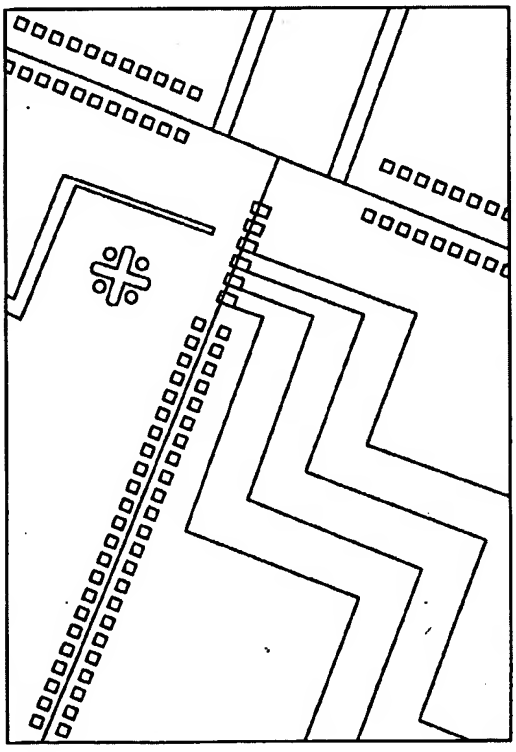


FIG.30B

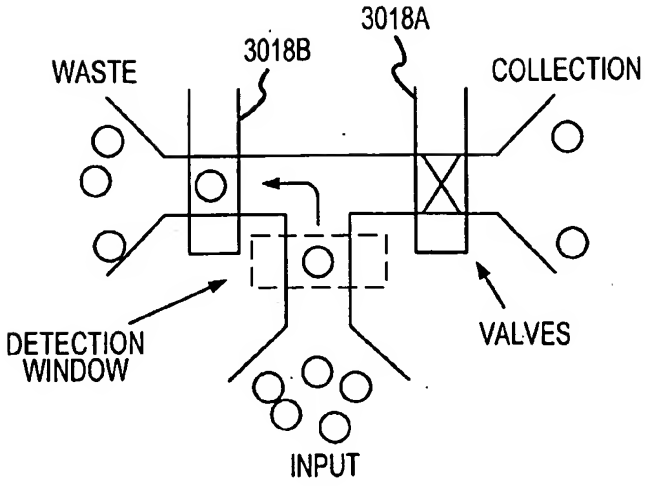


FIG.31A

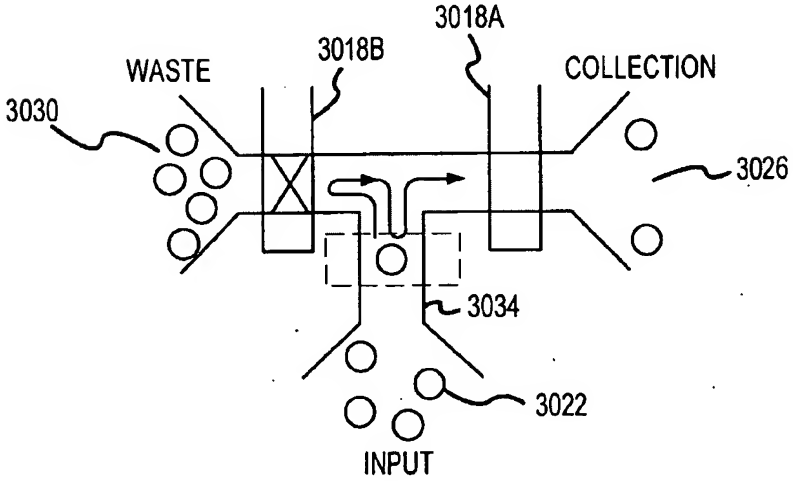


FIG.31B

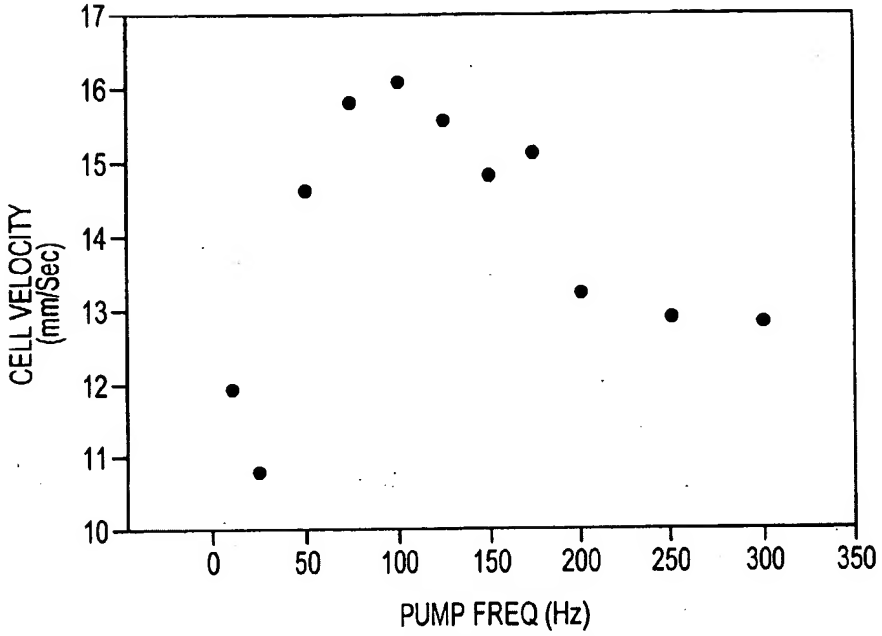


FIG.32A

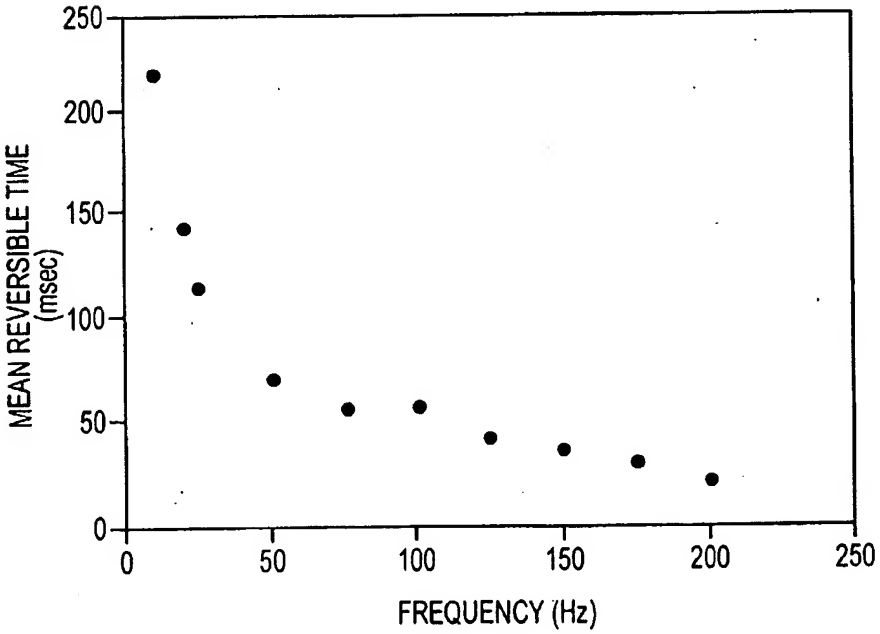


FIG.32B

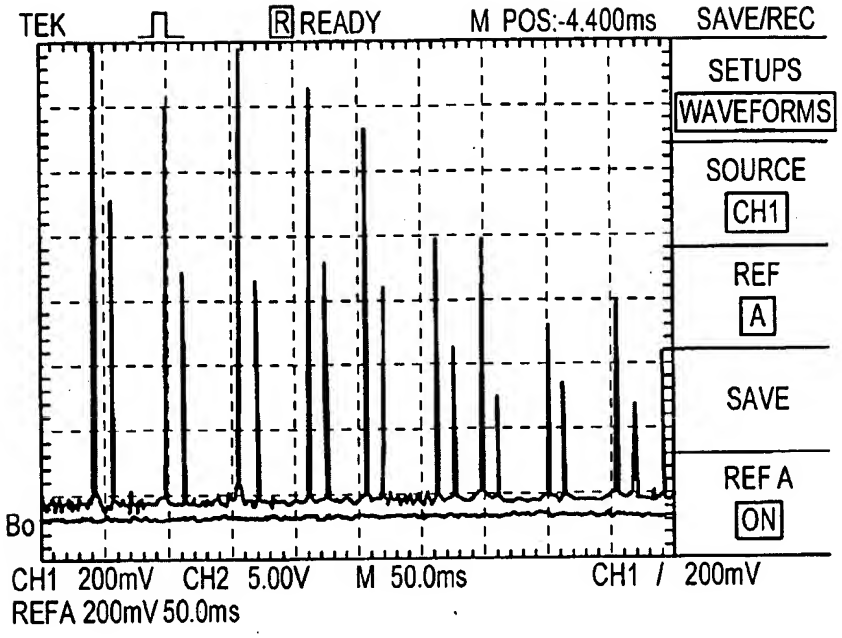


FIG.33A

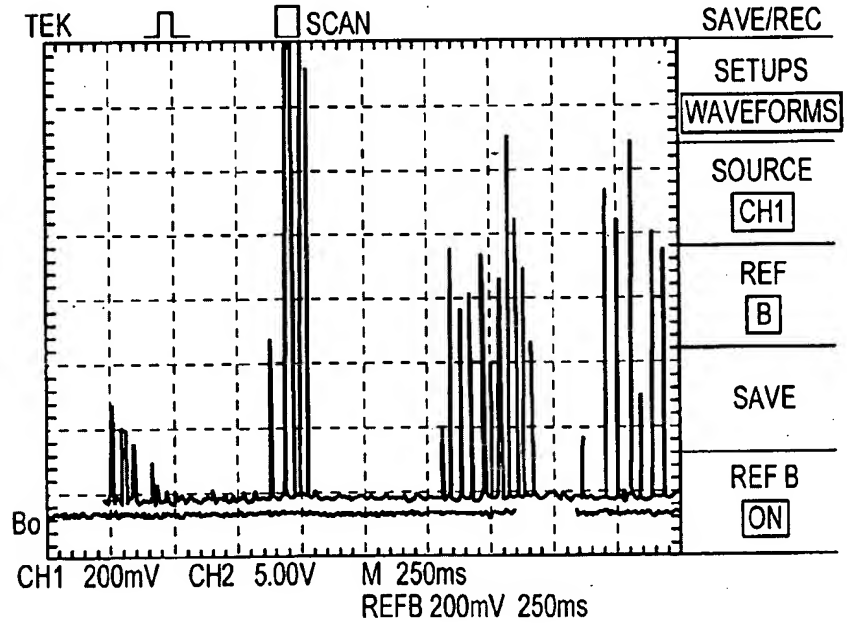


FIG.33B

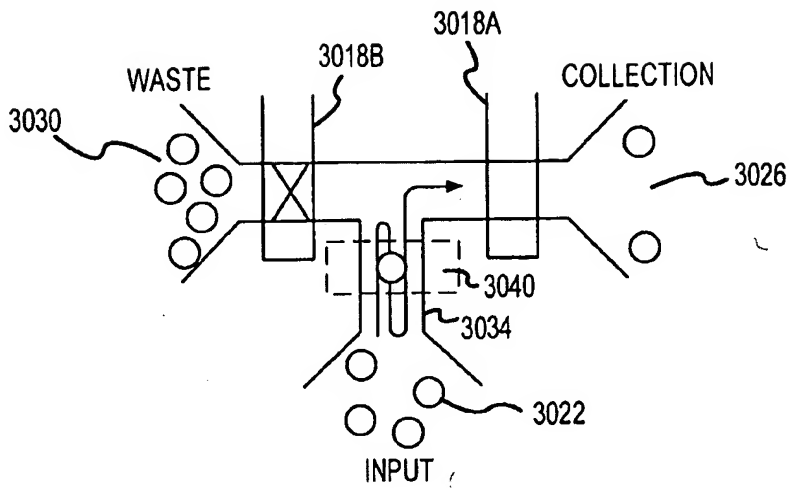


FIG.33C

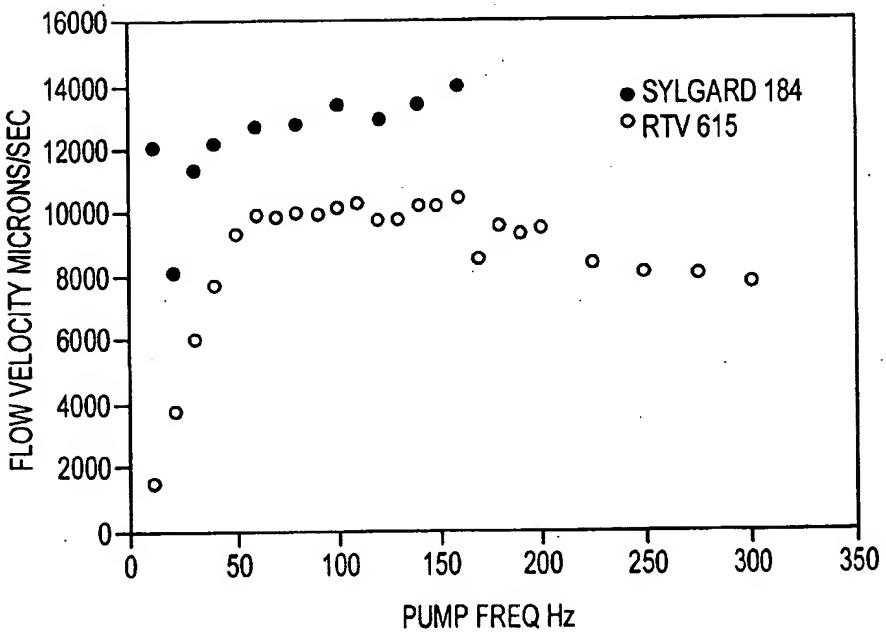


FIG.34

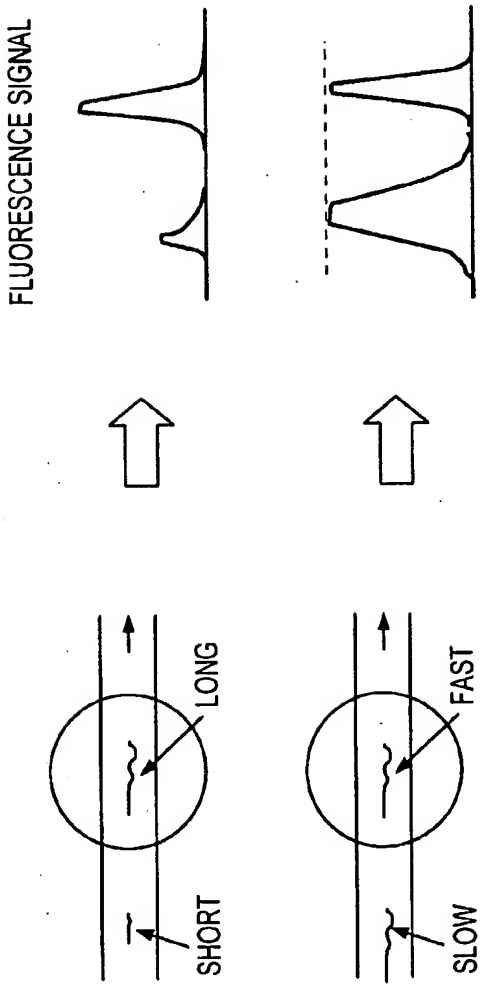


FIG.35A
FIG.35B
FLUORESCENCE SIGNAL
LONG
SHORT
FAST
SLOW

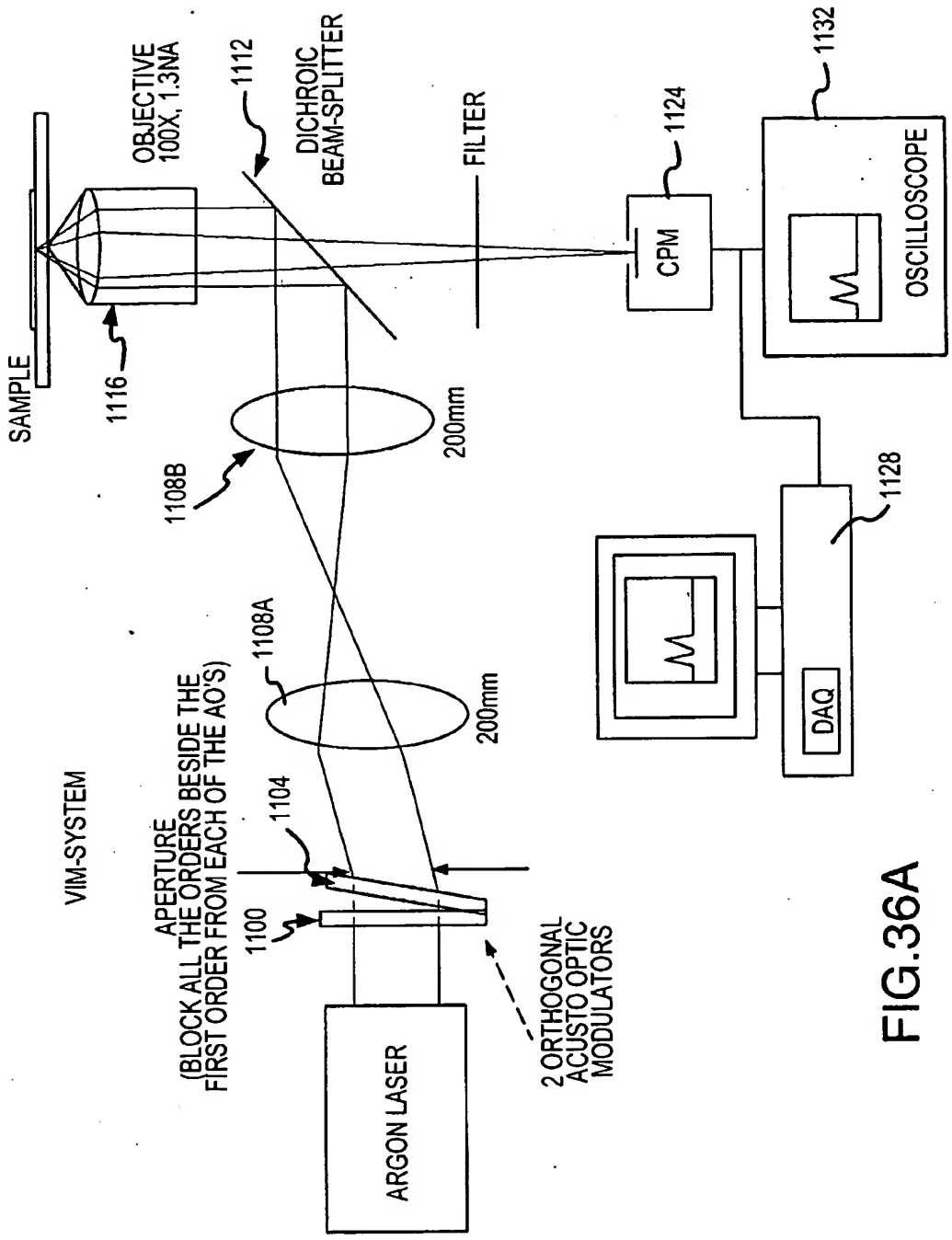

VIM-SYSTEM
ARGON LASER
2 ORTHOGONAL ACUSTO OPTIC MODULATORS
1100
APERTURE (BLOCK ALL THE ORDERS BESIDE THE FIRST ORDER FROM EACH OF THE AO'S)
1104
1108A
200mm
1108B
200mm
SAMPLE
1116
OBJECTIVE 100X, 1.3NA
1112
DICHROIC BEAM-SPLITTER
FILTER
CPM
1124
1132
OSCILLOSCOPE
1128
DAQ

FIG.36A

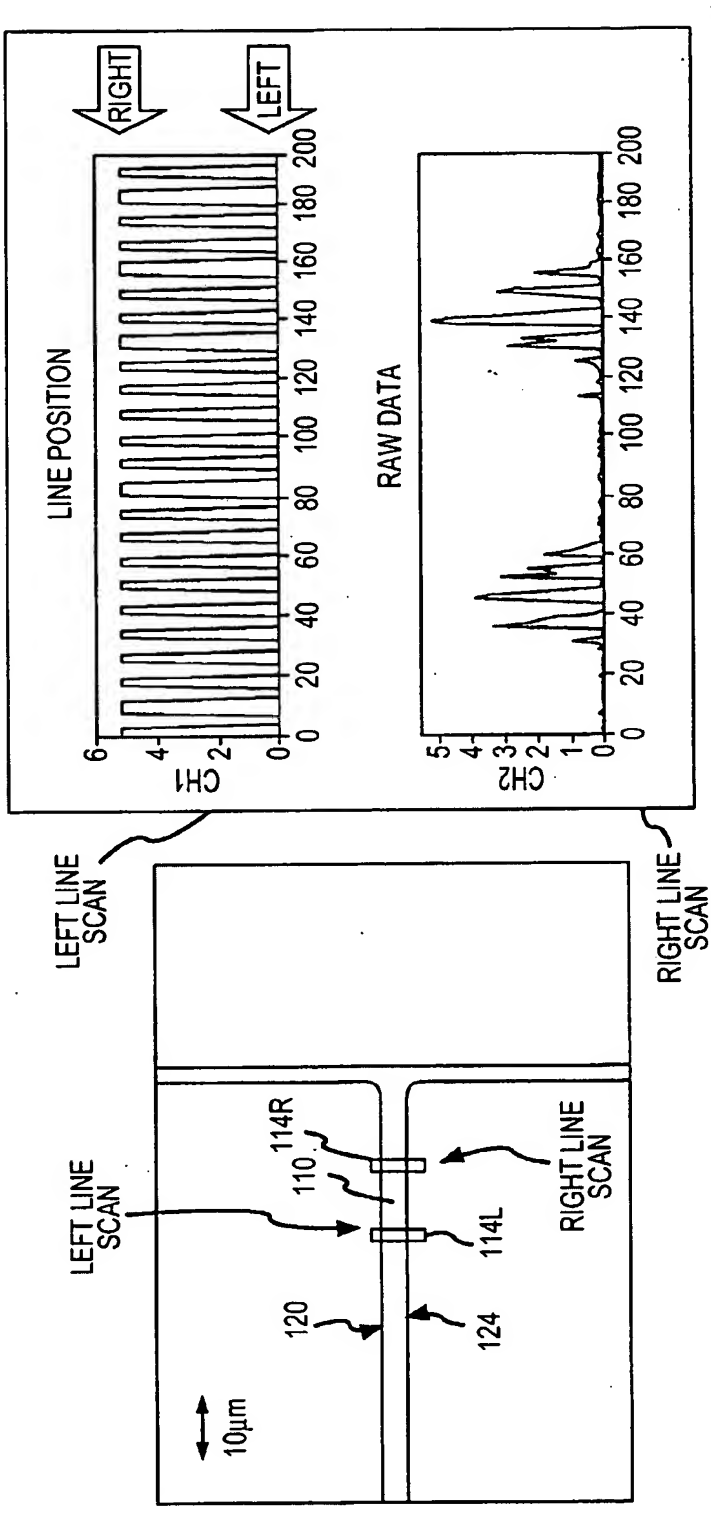

LINE POSITION
RIGHT
LEFT
CH1
RAW DATA
CH2
LEFT LINE SCAN
RIGHT LINE SCAN
114R
110
114L
120
124
10µm

FIG.36B

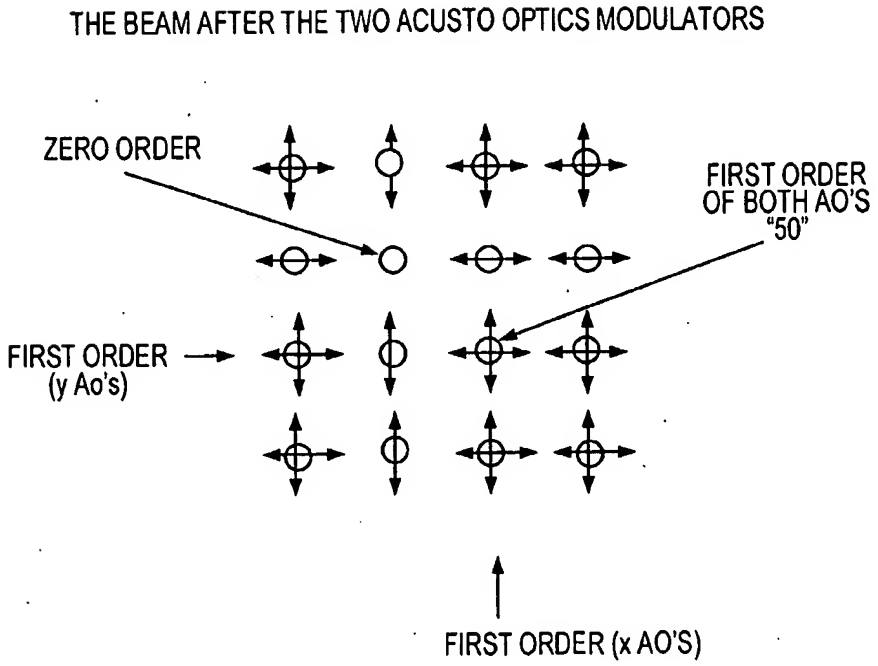

THE BEAM AFTER THE TWO ACUSTO OPTICS MODULATORS
ZERO ORDER
FIRST ORDER OF BOTH AO'S "50"
FIRST ORDER (y Ao's)
FIRST ORDER (x AO'S)

FIG.36C

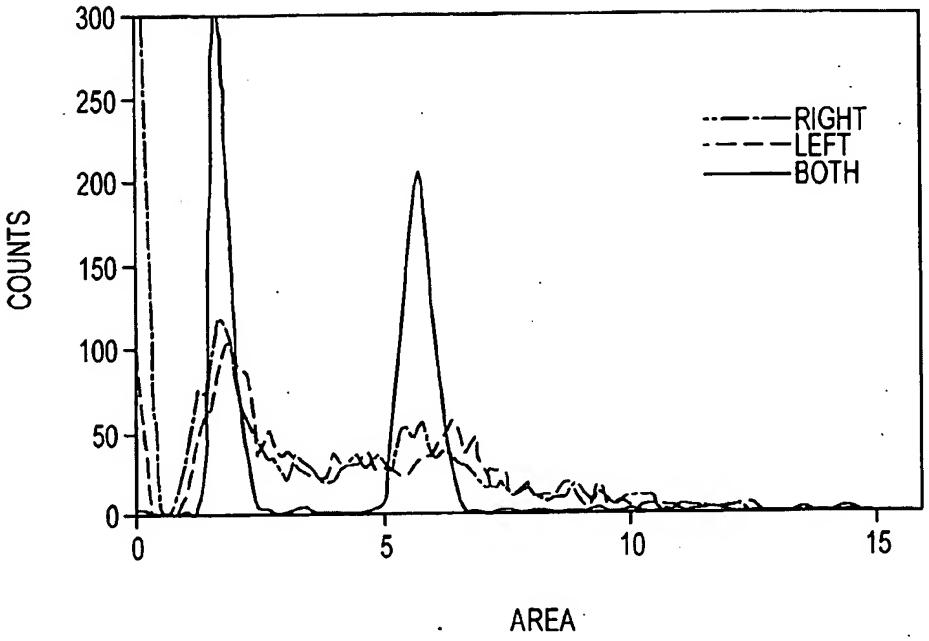

300
250
200
150
100
50
0
COUNTS
0
5
10
15
AREA
RIGHT
LEFT
BOTH

FIG.37

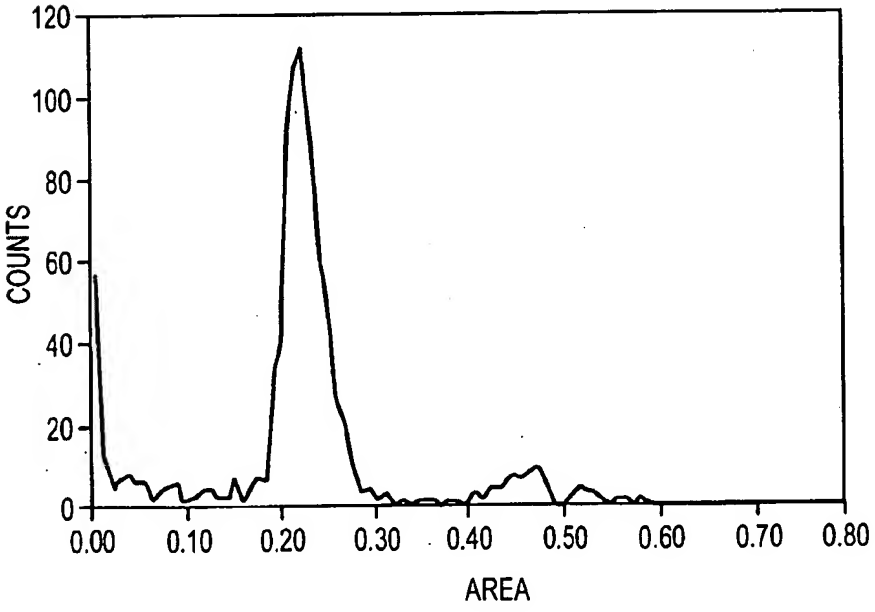

120
100
80
60
40
20
0
COUNTS
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
AREA

FIG.40

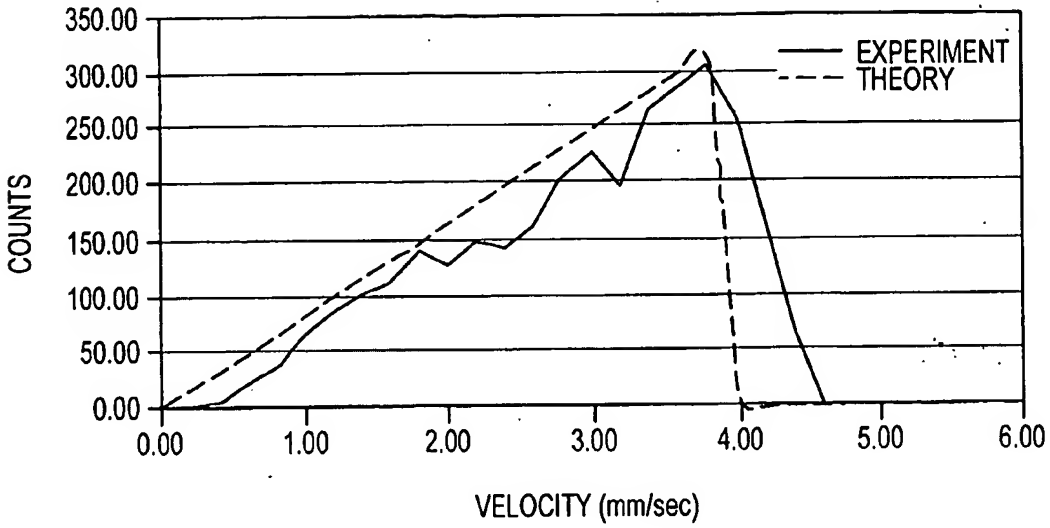

350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
COUNTS
EXPERIMENT
THEORY
0.00
1.00
2.00
3.00
4.00
5.00
6.00
VELOCITY (mm/sec)

FIG.39

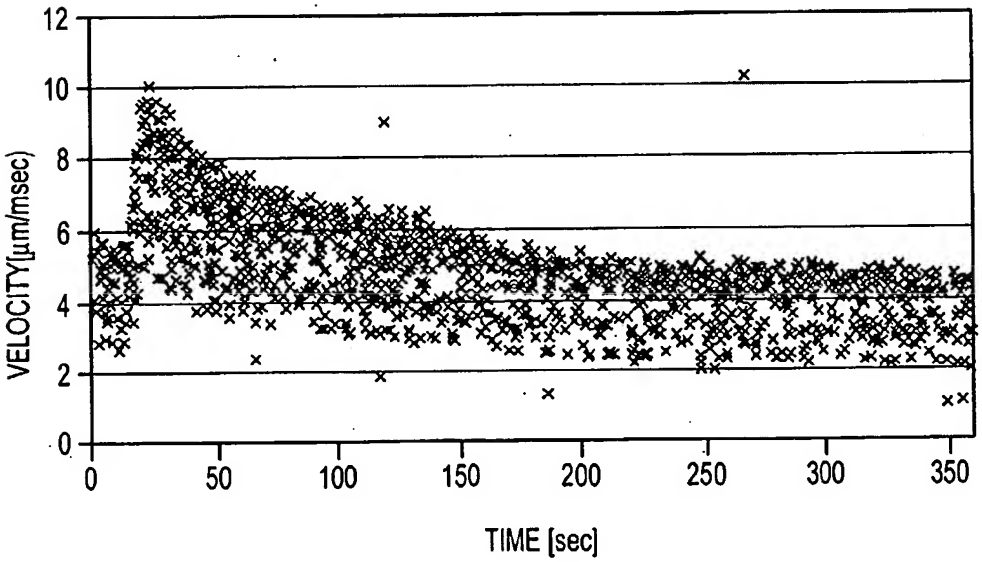

12
10
8
6
4
2
0
VELOCITY(μm/msec)
0
50
100
150
200
250
300
350
TIME [sec]

FIG.38

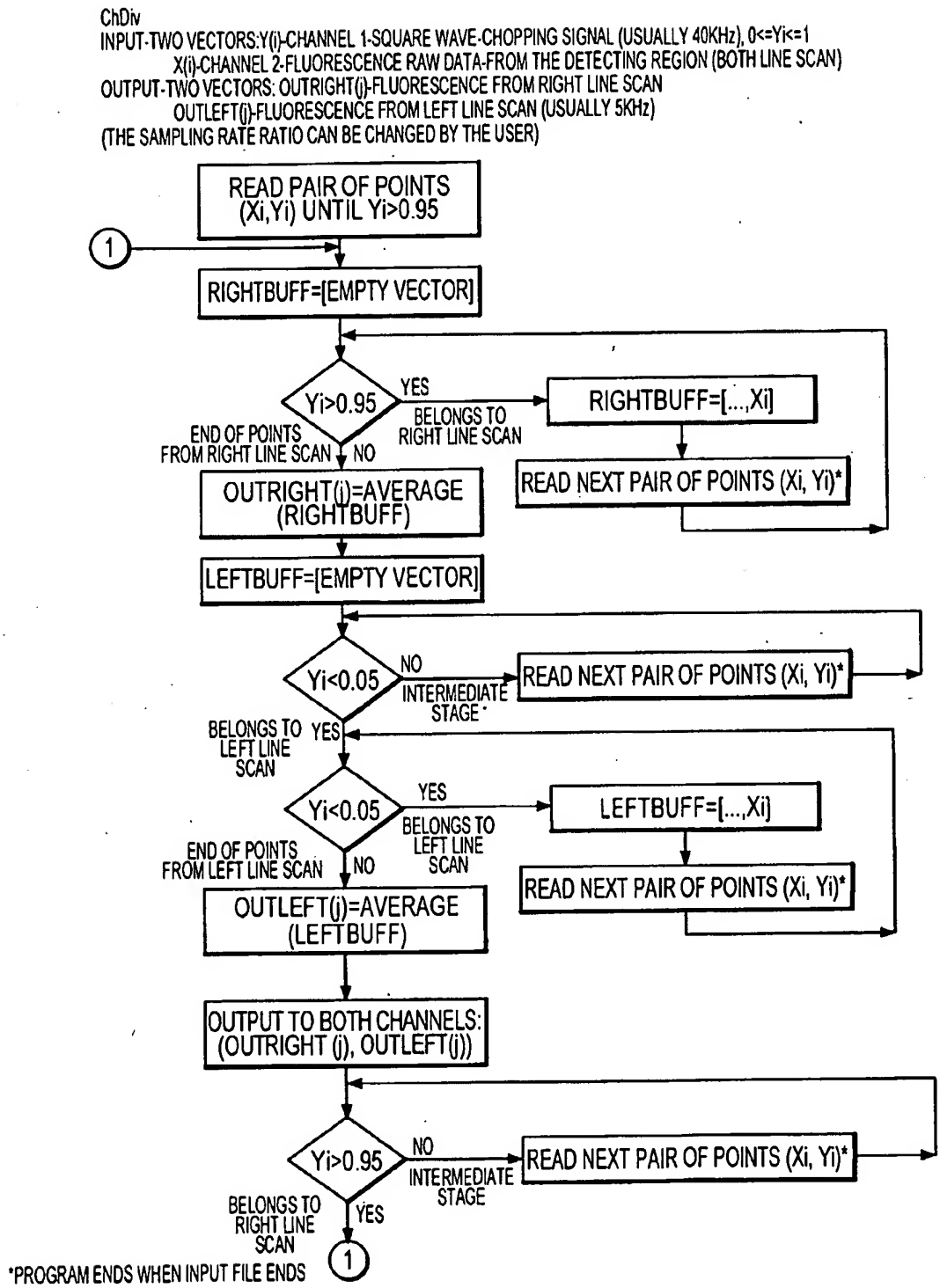
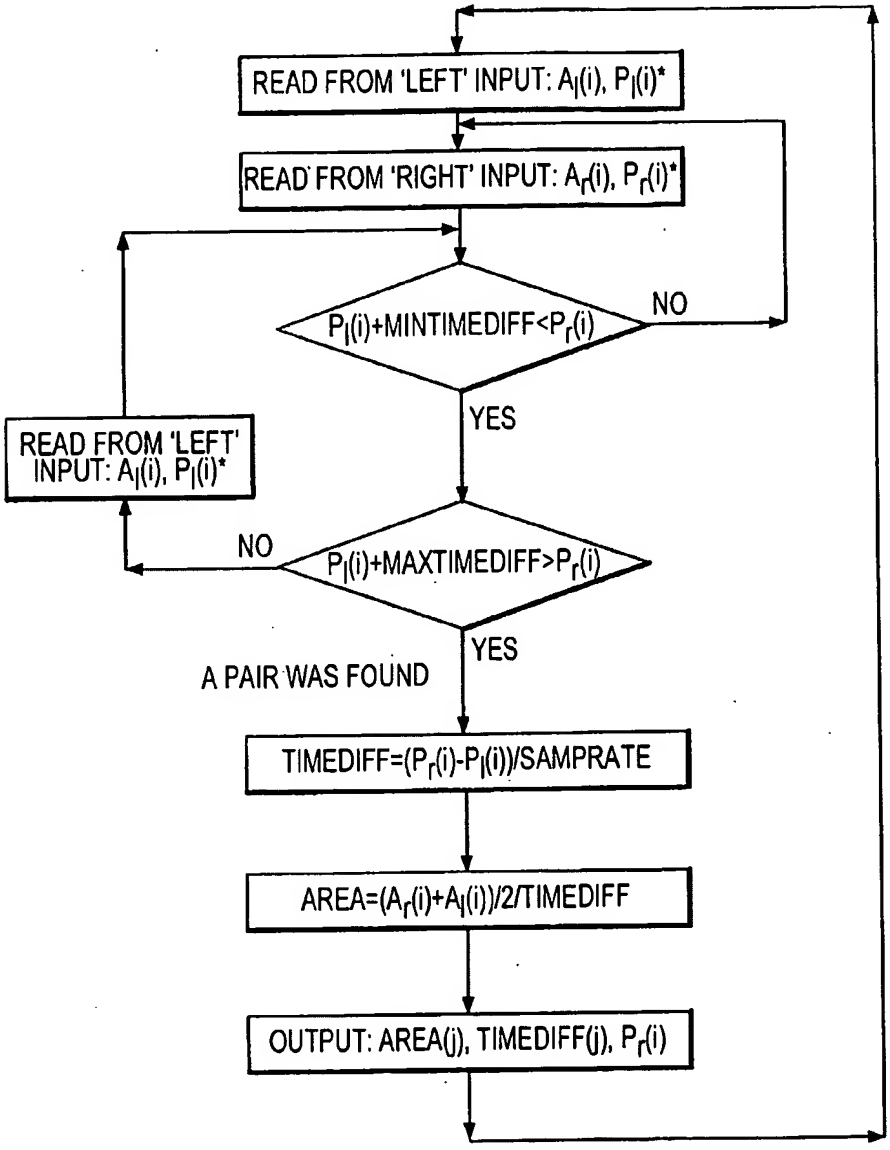

ChDiv
INPUT-TWO VECTORS:Y(i)-CHANNEL 1-SQUARE WAVE-CHOPPING SIGNAL (USUALLY 40KHz), 0<=Yi<=1
X(i)-CHANNEL 2-FLUORESCENCE RAW DATA-FROM THE DETECTING REGION (BOTH LINE SCAN)
OUTPUT-TWO VECTORS: OUTRIGHT(j)-FLUORESCENCE FROM RIGHT LINE SCAN
OUTLEFT(j)-FLUORESCENCE FROM LEFT LINE SCAN (USUALLY 5KHz)
(THE SAMPLING RATE RATIO CAN BE CHANGED BY THE USER)
READ PAIR OF POINTS (Xi,Yi) UNTIL Yi>0.95
1
RIGHTBUFF=[EMPTY VECTOR]
Yi>0.95
YES
BELONGS TO RIGHT LINE SCAN
RIGHTBUFF=[...,Xi]
READ NEXT PAIR OF POINTS (Xi, Yi)*
END OF POINTS FROM RIGHT LINE SCAN
NO
OUTRIGHT(j)=AVERAGE (RIGHTBUFF)
LEFTBUFF=[EMPTY VECTOR]
Yi<0.05
NO
INTERMEDIATE STAGE
READ NEXT PAIR OF POINTS (Xi, Yi)*
BELONGS TO LEFT LINE SCAN
YES
Yi<0.05
YES
BELONGS TO LEFT LINE SCAN
LEFTBUFF=[...,Xi]
READ NEXT PAIR OF POINTS (Xi, Yi)*
END OF POINTS FROM LEFT LINE SCAN
NO
OUTLEFT(j)=AVERAGE (LEFTBUFF)
OUTPUT TO BOTH CHANNELS: (OUTRIGHT (j), OUTLEFT(j))
Yi>0.95
NO
INTERMEDIATE STAGE
READ NEXT PAIR OF POINTS (Xi, Yi)*
BELONGS TO RIGHT LINE SCAN
YES
1
*PROGRAM ENDS WHEN INPUT FILE ENDS

FIG.41

INPUT: TWO FILES (ONE FOR EACH LINE SCAN).
EACH FILE CONTAIN 2 VECTORS ONE OF POSITIONS (P(i)) AND THE OTHER HAS THE CORRESPONDING AREA (A(i))
OUTPUT: THREE VECTORS-AREA, TIMEDIFF (INVERSELY PROPORTIONAL TO VELOCITY), POSITION
PARAMETERS THAT CAN BE DETERMINED-MINTIMEDIFF, MAXTIMEDIFF



POSITION IS PRESENTED IN POINT NUMBER AND NOT TIME
TIMEDIFF IS IN SECONDS AND IS INVERSELY PROPORTIONAL TO THE VELOCITY
*PROGRAM ENDS WHEN ONE OF THE INPUT FILES ENDS

FIG.42